POSITIONAL CLONING OF THE *GUNMETAL* MOUSE MUTATION

By

JOHN C. DETTER

A DISSERTATION PRESENTED TO THE GRADUATE SCHOOL
OF THE UNIVERSITY OF FLORIDA IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

UNIVERSITY OF FLORIDA

1999

This work is dedicated to my late father with loving memories, as well as to my vigorously young-at-heart mom and my forever-loving wife.

ACKNOWLEDGMENTS

The author would like to thank his mentor, Dr. Stephen F. Kingsmore, his co-mentor, Dr. Margaret R. Wallace, his supervisory committee members--Dr. Camilynn I. Brannan, Dr. Daniel J. Driscoll, and Dr. Wayne T. McCormack--and his indispensable collaborator, Dr. Richard T. Swank, for their valuable advice and encouragement.

The author's appreciation is extended to all former and present members of Dr. Kingsmore's laboratory--Dr. Vishnu S. Mishra, Dr. Velizar T. Tchernev, Dr. Elizabeth B. McMurtrie, Dr. Maria D.F.S. Barbosa, Quan A. Nguyen, Juan M. Teodoro, and Sandra M. Holt--who contributed to the successful completion of this work.

The author would like especially to acknowledge Dr. Elizabeth B. McMurtrie, who significantly aided in the initial stages of this work and helped to keep the author sane. The author must also recognize and thank his wife, Sara Detter, for putting up with the author's exasperating behavior at times, as well as his measly income.

TABLE OF CONTENTS

page

ACKNOWLEDGMENTS iii

LIST OF TABLES vi

LIST OF FIGURES vii

ABSTRACT ix

CHAPTERS

1 INTRODUCTION 1

Megakaryocytes and Platelets. 1
Gunmetal 5
Gray Platelet Syndrome 11
The Aim 12
The Significance 14
Initial Studies Toward First Specific Aim 15

2 IDENTIFICATION OF NOVEL SIMPLE SEQUENCE LENGTH POLYMORPHISMS (SSLP) IN MOUSE BY INTERSPERSED REPETITIVE ELEMENT (IRE)-PCR 18

Introduction 18
Materials and Methods 20
Results and Discussion 22

3 ISOLATION OF MOUSE TRANSCRIBED SEQUENCES FROM YAC/BAC CLONES USING AN INTERSPERSED REPETITIVE ELEMENT (IRE)-PCR BASED DIRECT cDNA SELECTION APPROACH 25

Introduction 25
Materials and Methods 27
Results and Discussion 31

4 RAB GERANYLGERANYL TRANSFERASE MUTATION IN THE *GUNMETAL* MOUSE CAUSES DEFICIENT RAB27a PRENYLATION AND PLATELET SYNTHESIS 33

Introduction 33
Materials and Methods 35
Results and Discussion 40

5 CONCLUSIONS AND FUTURE DIRECTIONS 55

Summary of Results 56
Discussion 60
Future Directions 62

APPENDICES

A MOUSE *RABGGTA* SEQUENCES SUBMITTED TO GENBANK 67

B INTER-SPECIES *RABGGTA* SEQUENCE AND AMINO ACID COMPARISONS 90

REFERENCES 99

BIOGRAPHICAL SKETCH 105

LIST OF TABLES

Table page

2.1 Novel simple sequence length polymorphisms.....23

3.1 Isolated direct cDNA selection products.....30

LIST OF FIGURES

Figure page

1.1 Transmission electron micrographs of *gm/+* and *gm/gm* platelets.....6

1.2 Transmission electron micrograph of a *gm/+* and *gm/gm* megakaryocyte. ...8

1.3 Abnormal expression of LMW GTP-binding proteins in (*gm/gm*) platelets. ...9

1.4 Flow diagram that summarizes the strategy. 13

1.5 Preliminary *gm* genetic mouse maps. 15

2.1 Consensus sequence of the B1 repetitive element. 19

2.2 Flow diagram of the IRE-PCR method. 20

3.1 Schematic representation of the IRE-PCR modified direct cDNA selection procedure. 28

4.1 Mouse chromosome 14 haplotype analysis of three *gm* backcrosses.....41

4.2 Mouse chromosome 14 genetic ideograms of the three *gm* backcrosses...42

4.3 Genetic and physical maps of mouse Chr 14 in the vicinity of *gm*.....43

4.4 A splice acceptor mutation in *Rabggta* causes abnormal splicing. 45

4.5 Decreased Rabggta protein levels in *gm/gm* mice. 48

4.6 Decreased Rab GGTase activity in *gm/gm* mice. 50

4.7 Deficient prenylation of Rab27a in *gm* platelets. 51

4.8 Deficient prenylation of Rab27a in *gm* platelets. 53

4.9 Differential intracellular localization of Rab27a in *gm* platelets compared to wild-type platelets. 53

5.1 Diagrammatic representation of Rabggta's role in Rab prenylation and membrane attachment.....59

Abstract of Dissertation Presented to the Graduate School
of the University of Florida in Partial Fulfillment of the
Requirements for the Degree of Doctor of Philosophy

POSITIONAL CLONING OF THE *GUNMETAL* MOUSE MUTATION

By

John C. Detter

August 1999

Chairman: Stephen F. Kingsmore
Major Department: Molecular Genetics and Microbiology

Few molecular events important in platelet biogenesis have been identified. Mice homozygous for the recessive mutation gunmetal (*gm*) have platelet and megakaryocyte defects that cause prolonged bleeding and high mortality; *gm* platelets are increased in size, decreased in abundance, lack α- and δ-granule proteins, and contain abnormal small GTPases; *gm* megakaryocytes are increased in abundance and have abnormal intracellular membrane accumulations. The main goal of this dissertation work was to identify the gene responsible for the *gm* mouse mutant using a positional cloning strategy. Positional cloning is an approach to disease gene identification based primarily on genetic location. The steps involved include: (1) genetic localization; (2) physical contig assembly; (3) candidate gene identification; (4) mutation analysis; and (5) characterization of gene mutation.

Using 2462 *gm* backcross mice, we localized *gm* to a 0.64 centiMorgan (cM) region of mouse chromosome (Chr) 14. Using direct cDNA selection, the α-subunit of Rab geranylgeranyl transferase (*Rabggta*) was isolated from contiguous yeast artificial chromosome (YAC) and bacterial artificial chromosome (BAC) clones that contained this region. In *gm* mice, DNA sequence analysis of *Rabggta* identified a G → A substitution at residue -1 of the intronic splice acceptor site preceding exon 1 (which contains the start codon). Most *gm Rabggta* mRNAs lacked the start codon due to exon 1 skipping, while a minute amount retained the start codon due to the utilization of cryptic splice sites. Rab geranylgeranyl transferase (Rab GGTase, composed of α, β, and REP-1 components) covalently attaches lipid moieties to the C-terminus of Rab GTPases, enabling reversible attachment to membranes. Rabs regulate intracellular vesicular transport by acting as molecular switches that oscillate between membrane-attached, GTP-bound and cytosolic, GDP-bound states. Levels of Rabggta protein and Rab GGTase activity were reduced 4-fold in *gm* platelets. Prenylation and membrane association of a Rab GGTase substrate, Rab27a, was also decreased in *gm* platelets. These data indicate that prenylation of Rab GTPases is important for platelet synthesis and delivery of proteins to platelet granules, and suggest Rab GGTase as a therapeutic target for thrombocytosis and clotting disorders.

CHAPTER 1
INTRODUCTION

Despite numerous studies, relatively few molecular mechanisms or biochemical pathways regulating platelet production by megakaryocytes are known. This is a significant deficiency, since inherited and acquired platelet defects are not uncommon, and currently are treated with repeated platelet transfusions, which are very costly, may transmit infectious diseases, and may result in alloimmunization.[1,2] Identification of the molecular basis of Mendelian mouse models of platelet defects is an effective way to identify new and important pathways or factors involved in platelet production from megakaryocytes.

Megakaryocytes and Platelets

Hematopoiesis involves the differentiation of a bone marrow pluripotent stem cell into various cellular components of the blood. The megakaryoblast is one of the cell types that differentiates from the stem cell. With the aid of certain signals, this cell gives rise to the megakaryocyte and ultimately to 2000-8000 platelets (thrombocytes).[1,3]

Megakaryocyte maturation is marked by an increase in size, development of organelles, and acquisition of platelet proteins such as glycoprotein IIb/IIIa, thrombospondin (TSP), von Willebrand factor (vWF), and platelet factor 4 (PF4). The following outlines the stages and factors involved in megakaryocytopoiesis:

Stages of Megakaryocytopoiesis[1]

- *BFU-MK:* The burst-forming unit-megakaryocyte is the most undifferentiated committed megakaryocyte progenitor cell. It has great proliferative potential and can divide to produce many CFU-MKs.
- *CFU-MK:* The colony forming unit-megakaryocyte is a more differentiated progenitor cell than BFU-MK, but contains low proliferative potential.
- *Megakaryoblast:* The maturational events of megakaryocytopoiesis are thought to start with this mononuclear cell type. These cells, representing ~5% of all megakaryocytic elements in the marrow, are characterized by their small size and round morphology. They also express phenotypic markers belonging to the megakaryocyte, such as proteins contained in the α-granule and membrane glycoproteins.[1]
- *Megakaryocyte:* This is the final developmental stage. As megakaryocytes proliferate, they enlarge and undergo extensive DNA replication without mitosis. The polyploid cells subsequently undergo endomitosis to form multilobed nuclei with four to 64 times the haploid amount of DNA, most commonly 16 or 32N.[4] The mature megakaryocyte is located directly adjacent to bone marrow sinusoidal endothelial cells. As extensive

demarcation membranes form by invaginations of the plasma membrane, megakaryocytes develop long filopodia that directly penetrate the endothelial cytoplasm and extend into the marrow capillaries.[5] These projections then fragment to produce mature thrombocytes (platelets). More than 2000 platelets are ultimately produced from the cytoplasm of a single mature megakaryocyte.[4]

Factors Involved in Megakaryocytopoiesis[1]

- *Proliferative/Growth factors* = thrombopoietin (TPO), IL-3, & GM-CSF
- *Maturative/Differentiation factors (cell compartment factors)* = TPO, IL-6, & 11
- *Inhibitory factors* = platelet factor 4 (PF4)

Platelets are very small, anucleate cells that contain localized concentrations of molecules required for hemostasis, the stopping of a flow or circulation of blood. Platelets are heterogeneous in size and reactivity, and large platelets are more hemostatically active than small platelets.[6] Platelets must undergo three processes for hemostasis to occur: **adhesion** to exposed collagen in blood vessels; **release** of the contents of their granules; and **aggregation,** as described below.[7]

1. Platelet *adhesion* to collagen on the damaged vessel wall is the first step in hemostasis and thrombosis, the development of a clot. This activation process is mediated by von Willebrand's factor (vWF), a glycoprotein secreted by endothelial cells and platelets into plasma. The vWF protein

acts as a secure bridge between a glycoprotein complex on platelet surfaces and collagen fibrils in the walls of blood vessels. It thus prevents platelets from being detached from vessel walls by high shearing forces developed in the blood vessels.[8]

2. Once the platelets are activated they change shape and *release* the contents of their alpha and dense granules to increase activation and promote aggregation. The alpha (α) granules are distinct storage organelles containing a plethora of proteins, such as vWF, fibronectin, and thrombospondin. When released, these proteins take part in platelet reactions during adhesion, aggregation, and clot formation.[9] Dense (δ) granules differ from α-granules in that their electron-dense contents may fill the whole or the central matrix of the organelle. The components of δ-granules (ADP, Ca^{2+}, and serotonin) aid in platelet aggregation and transmembrane signaling. Granule release reaction disorders are known as storage pool diseases (SPDs). SPD of the α-granules include gray platelet syndrome while SPD of the δ-granules involve Hermansky-Pudlak syndrome and Chediak-Higashi syndrome.[10] SPD of both α- and δ-granules together (alpha/delta SPD) has also been reported.[11]
3. Increased platelet activation with thrombin and ADP allows interplatelet contact and the formation of platelet *aggregates*. ADP modifies the platelet surface so that fibrinogen will attach to a complex of two glycoproteins on the platelet surface. Molecules of fibrinogen then link

adjacent platelets to each other, forming a platelet aggregate.[12] The aggregate structure results in an impermeable multicellular thrombocyte thrombus (clot) that eventually releases tissue plasminogen activator (TPA) to prevent complete blockage of the vessel.[8]

The above processes, megakaryocytopoiesis and thrombopoiesis, are thought to be under the control of a feedback mechanism involving platelet contents as the source of inhibitory factors for megakaryocyte progenitor cell growth.[1,13] As platelet numbers increase, inhibition of megakaryocyte progenitor cell growth increases, resulting in fewer megakaryocytes and thus a decrease in platelets. A low platelet count would reduce megakaryocyte inhibitory factors, and thus megakaryocyte numbers would increase, resulting in more platelets.

Gunmetal

A single-gene mutation in the mouse that affects platelet production is the pigment mutation gunmetal (*gm*). It is an autosomal recessive mutation that arose spontaneously in the C57BL/6J strain in 1962.[14] It was initially localized to central mouse Chromosome 14.[15] Gunmetal homozygotes (*gm*/*gm*) display a syndrome of partial albinism and platelet defects that is associated with prolonged bleeding, high mortality, and poor fecundity.

Characteristics of *gm/gm* Megakaryocytes and Platelets

Gunmetal homozygotes suffer from macrothrombocytopenia, a decrease in number and increase in size of blood platelets (Figure 1.1). *gm/gm* mice have only 40% of the normal platelet count, whereas *gm/+* mice have a normal platelet count. This deficiency is due to a 70% reduction in platelet synthesis in *gm* homozygotes compared to normal mice.[16] *gm/gm* mice also exhibit abnormal heterogeneity in platelet size. Many of the platelets observed in gunmetal mice are significantly larger than in normal mice.

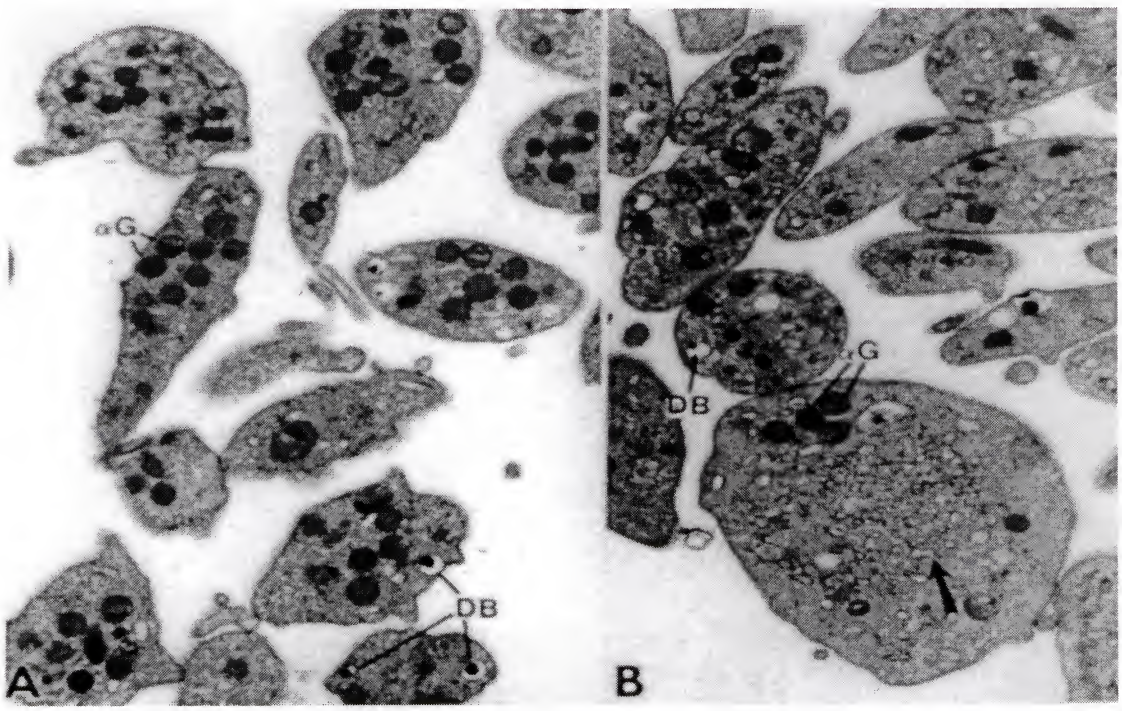

Figure 1.1: Transmission electron micrographs of *gm/+* and *gm/gm* platelets (adapted from Novak *et al.*, 1995). (A) The *gm/+* mouse platelets are normal in size, shape, and appearance. OM x 16,000. (B) The *gm/gm* mouse platelets show greater size heterogeneity, and some contain membrane complexes (arrow). OM x 16,000. DB, dense body; αG, alpha granule.

Gunmetal homozygotes display several dense granule abnormalities, thus laying the foundation for a mild δ-SPD. The *gm/gm* serotonin component of dense granules is only 50% of normal.[16] Additionally, the number of dense granules per *gm/gm* platelet is also 50% of normal.[16] Because of the larger volume of *gm/gm* platelets, the above are minimal estimates.

Gunmetal homozygotes exhibit numerous alpha-granule abnormalities, the basis of a severe α-SPD. Three α-granule components (fibrinogen, vWF, and PF4) in platelets from *gm/gm* mice show significant quantitative reductions on Western blot when compared with normal mouse platelets.[16] These α-granule abnormalities suggest that *gm* may be an animal model for human gray platelet syndrome (see below).

Gunmetal homozygotes demonstrate an abnormal megakaryocyte count. A four-fold increase in megakaryocyte count was observed in *gm/gm* mice.[17] This could be the result of a feedback mechanism responding to the decreased platelet production.

Gunmetal homozygotes show signs of abnormal emperipolesis, a process in which other cells traffic through the cytoplasm of the megakaryocyte (Figure 1.2). This event is greatly increased in gunmetal homozygotes compared to normal mice.[17] It has been suggested that chemotactic factors released from leaky mutant megakaryocyte α-granules recruit other cells within the mutant megakaryocyte cytoplasm. This is likely because α-granule components are reduced in platelets but are increased in plasma of *gm/gm* mice.[17]

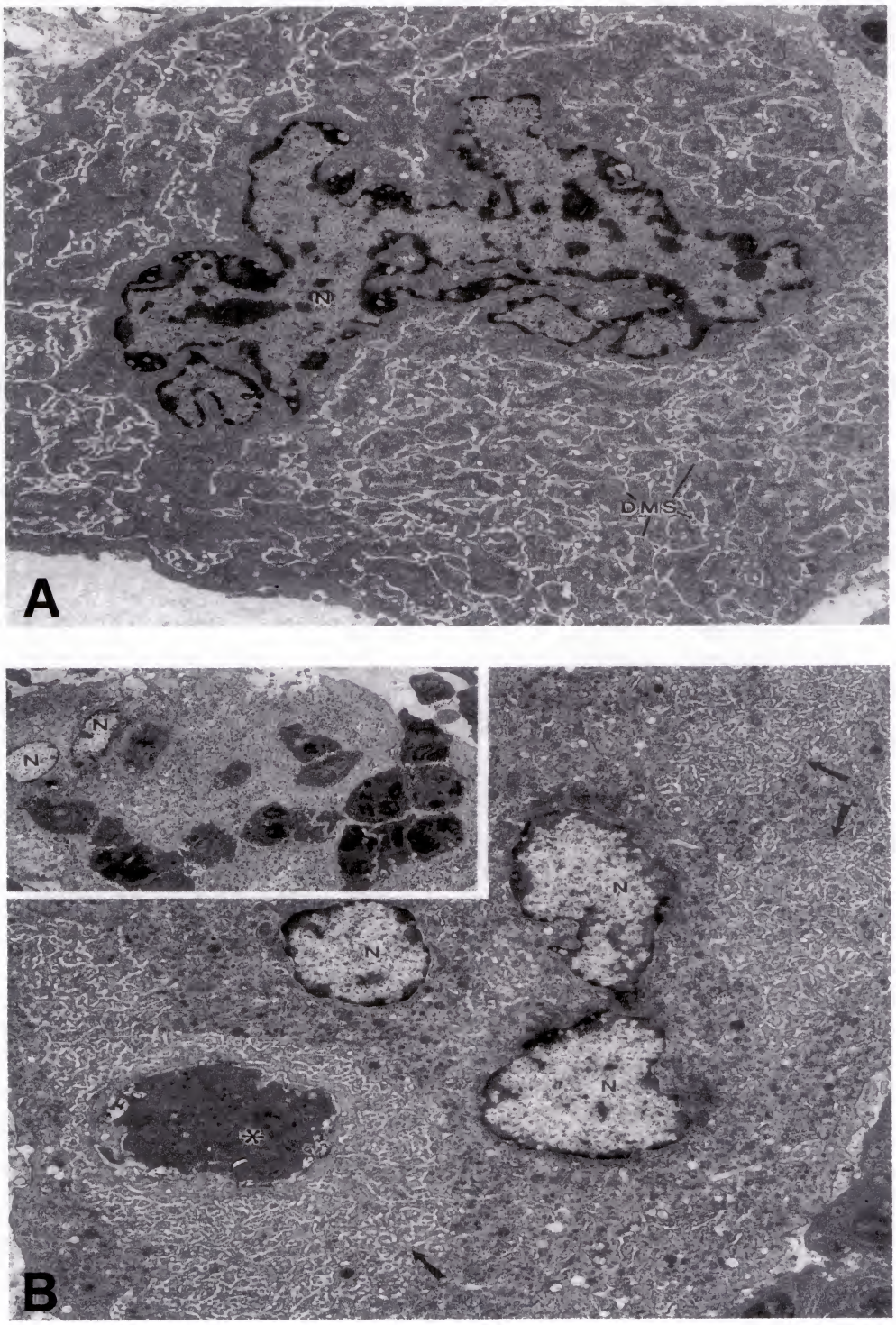

Figure 1.2: Transmission electron micrograph of a *gm/+* and *gm/gm* mouse megakaryocyte (adapted from Novak *et al.*, 1995). In contrast with the (A) *gm/+* normal megakaryocyte, the (B) *gm/gm* mutant megakaryocyte contains zones of membrane complexes (DMS, arrows) and an emperipolesed leukocyte (asterisk). The inset (B, top left) shows another cell from the same specimen that contains 17 emperipolesed cells. OM x 7,000; inset OM x 1,900; N, nucleus.

Gunmetal homozygotes display abnormal expression of two platelet low-molecular-weight GTP-binding proteins (Figure 1.3). Normal mouse platelets express 27.5, 26, & 23-Kd GTP-binding proteins. *gm/gm* mouse platelets express these three GTP-binding proteins as well as two novel (28.5 & 25-Kd) GTP-binding proteins.[16] The expression of these novel GTP-binding proteins is platelet specific and genetically recessive in nature.[16] GTP-binding proteins have been implicated in platelet formation due to their regulatory role in membrane budding and fusion reactions.[3,16,18]

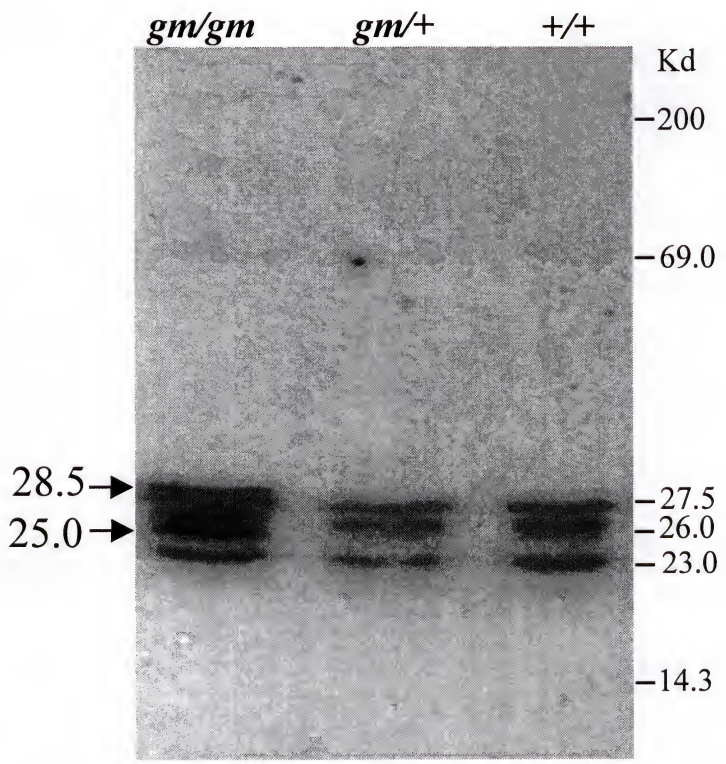

Figure 1.3: Abnormal expression of LMW GTP-binding proteins in platelets of gunmetal mice (adapted from Swank *et al.*, 1993). Platelet extracts from *gm/gm*, *gm/+*, and +/+ mice, equivalent in protein, were electrophoresed on SDS gels and Western-blotted to nitrocellulose membranes that were incubated with [^{32}P]GTP followed by autoradiography. The arrows indicate the 28.5 and 25.0-Kd GTP-binding proteins specific to *gm/gm* platelets.

Conclusions about the *gm* phenotype are as follows: (1) The macrothrombocytopenia suggests a low rate of platelet synthesis; (2) The reduction of several α-granule components in platelets suggests a general effect on organelle biogenesis rather than on specific α-granule components; (3) The high megakaryocyte count suggests a feedback mechanism is trying to counteract the low rate of platelet synthesis; (4) The deficiency of several α-granule proteins in the platelet α-granule and their appearance in plasma suggests an abnormality in targeting or retention of α-granule proteins rather than a defect in synthesis; (5) An abnormality in targeting could also account for the abnormal emperipolesis in *gm* megakaryocytes; (6) The novel platelet GTP-binding proteins may account for the platelet production deficiency based on the platelet activating capabilities of some GTP-binding proteins; (7) Additional evidence that shows reciprocal bone marrow transplantation between *gm/+* and *gm/gm* either corrected or caused abnormal platelet size and bleeding times,[17] suggests a defect in a marrow progenitor cell is responsible for the hematological defects associated with gunmetal mice.

Collectively, the characteristics of *gm* megakaryocytes and platelets suggest that the gunmetal gene acts intrinsically in megakaryocytes and that an abnormality in this gene causes significant qualitative and quantitative effects on platelet production.

Gray Platelet Syndrome

The most typical α-SPD in human is gray platelet syndrome (GPS).[10] Mild mucocutaneous bleeding tendency, moderate thrombocytopenia, large platelets, and incomplete aggregation responses to collagen, thrombin, or Ca^{2+} characterize this autosomal recessive human disorder.[19] Ultrastructural and cytochemical studies have shown that the platelets contain a normal number of dense granules but specifically lack α-granules. The platelet α-granule content (PF4, fibrinogen, TSP, fibronectin) is markedly decreased.[10] GPS megakaryocytes are also deficient in α-granules, however, plasma levels of α-granule proteins are increased. This suggests that GPS megakaryocytes can normally synthesize α-granule proteins but can not package them into precursors of the α-granule.[18]

Among inherited human bleeding diseases, gunmetal is most similar to GPS. Both have deficiencies of platelet α-granule proteins, reduced platelet numbers, and enlarged platelet volumes.[19] The appearance of platelet α-granule proteins in plasma is likewise common to both. GPS patients usually have a more marked deficiency of α-granule components and do not exhibit the δ-SPD seen in *gm* mice. However, the apparent δ-SPD difference between GPS and *gm* may be artifactual because δ-SPD in gunmetal is mild (only a twofold decrease in dense granule content) and because a similar twofold variation in platelet dense granule content is found among human controls.[19]

The Aim

Therefore, the main objective of this dissertation work was to positionally clone and characterize the gene responsible for the mouse mutation *gunmetal*, in order to gain insights into the biological processes involving megakaryocytopoiesis and thrombopoiesis. After the construction of two *gm* genetic backcrosses, the following specific aims for the present dissertation were determined (see flow diagram, Figure 1.4):

Specific Aim 1: Create a Precise Genetic Map of Mouse Chr 14 in Vicinity of *gm*

DNA from three large *gm* interspecific backcrosses will be PCR-amplified with polymorphic microsatellite markers (SSLP) to localize *gm* within an ~0.1cM interval on central mouse Chr 14.

Specific Aim 2: Generate a Physical Map Spanning the *gm* Critical Region

A yeast artificial chromosome (YAC) contig, with several-fold redundancy, of the *gm* nonrecombinant interval will be constructed by screening YAC libraries using genetic markers tightly linked to *gm*. New Sequence-Tagged Site (STS) will be derived if necessary and used with the initial genetic markers to STS content map YAC clones.

Specific Aim 3: Identify *gm* Candidate Genes Based on Location, Function, and Expression

cDNA selection will be used to isolate genes from YACs spanning the *gm* critical region. These genes will be examined for candidacy for *gm* based on genetic location, known function, and tissue distribution of expression.

Specific Aim 4: Screen *gm* Candidate cDNAs for Mutations

Genes considered favorable candidates for *gm* will be screened for mutations using RT-PCR, Northern blots, Southern blots, and sequence analysis from *gm* and normal mice.

Specific Aim 5: Characterize the *gm* Gene

Intense characterization of the *gm* gene requires full-length cDNA. *gm* cDNA clones will be isolated from bone marrow cDNA libraries until a full-length clone is assembled and sequenced. The full-length mouse clone will be used to isolate and map the homologous human gene. Molecular characterization of the *gm* gene will aid in the understanding of inherited human platelet disorders such as gray platelet syndrome.

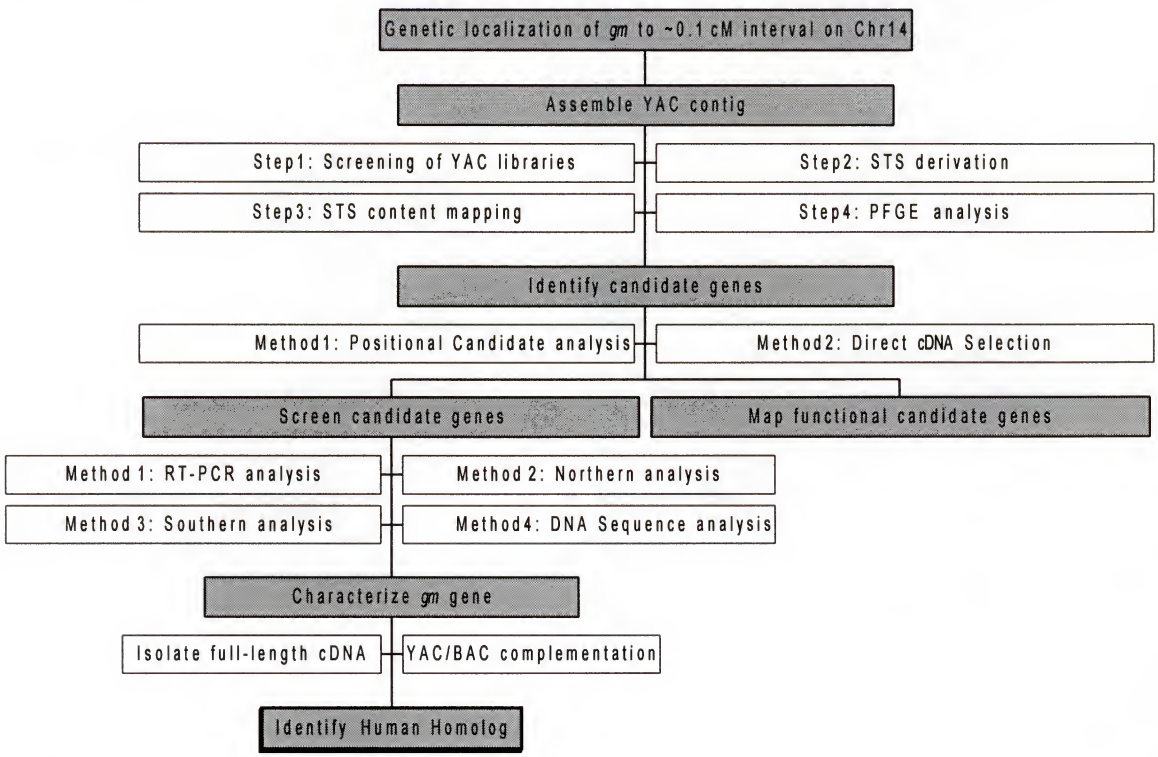

Figure 1.4: Flow diagram that summarizes the strategy.

The Significance

Platelet organelles and enzyme systems are created in the megakaryocyte, and changes in platelet volume and reactivity are correlated with and preceded by changes in the megakaryocyte.[1] Therefore, perturbations of megakaryocyte physiology may have important pathological implications for the development of atherosclerosis and thrombosis, which remain a major cause of morbidity and mortality in the Western world. Identification and analysis of the *gunmetal* gene is a unique opportunity to learn about new pathways and proteins involved in these processes. This project uses the power of mouse genetics and the fortuitous *gm* mutation to increase our understanding of megakaryocyte physiology. A better understanding of the biogenesis of platelet alpha and dense granules will contribute to treatments and cures for some of these devastating blood disorders by identifying affected biochemical pathways, thereby providing new and improved therapeutic targets.

Initial Studies Toward First Specific Aim

At the outset of our study, a precise genetic map in the vicinity of *gm* had been generated with the aid of our collaborators (Dr. Richard Swank, Roswell Park Cancer Inst., Buffalo, NY).[20] Two large interspecific backcrosses were produced in an attempt to maximize the chance of finding polymorphism in the *gm* region. The progeny were typed with many polymorphic microsatellites and genes in the vicinity of *gm* (Figure 1.5). *gm* was localized to central mouse Chr 14 by minimizing single crossover events and eliminating double crossover events.[21] The results are described below.

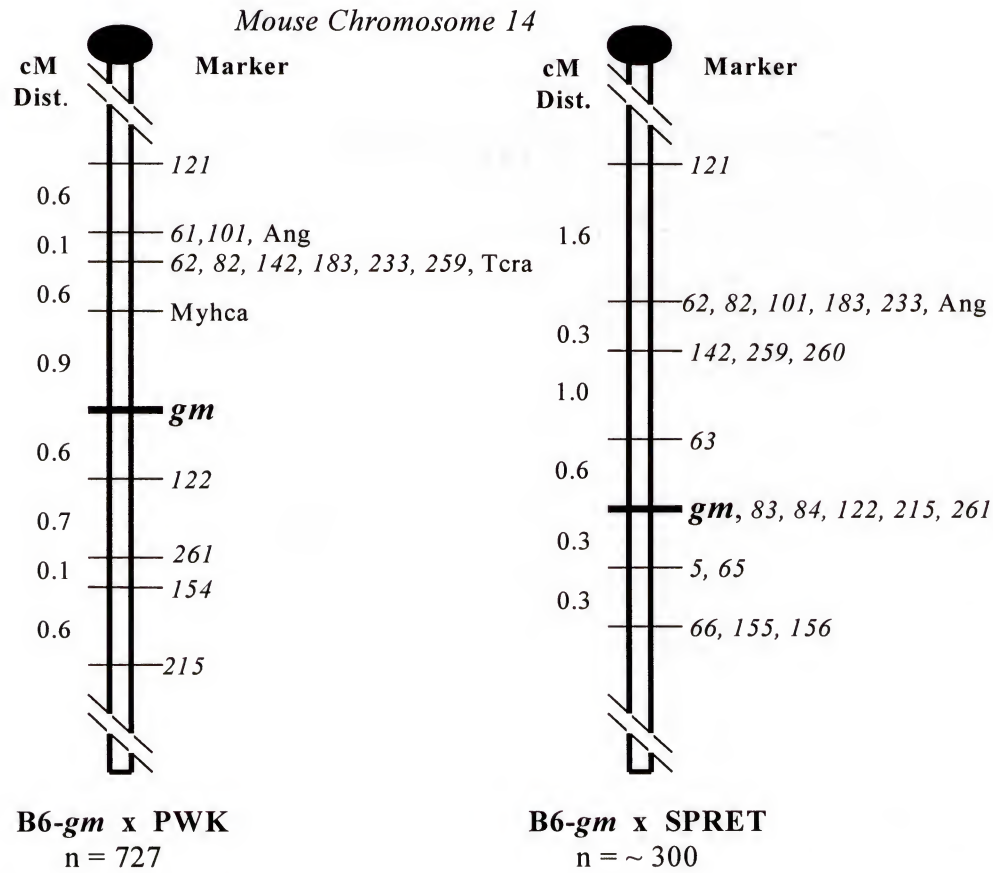

Figure 1.5: Preliminary *gm* genetic mouse maps.

Cross #1 Results: [(C57BL/6J-*gm* x PWK)F_1 X C57BL/6J-*gm*] = ~750 progeny[20]

- *gm* is localized to a 1.5cM interval.
- *gm* is flanked proximally by *Myhca* and distally by *D14Mit122*.
- No markers cosegregate with *gm*.
- This cross appears to have undergone a distance expansion (recombination amplification) and polymorphism decrease. The few markers that are polymorphic in this cross have genetically larger distances between them, compared to cross #2 and other published mapping results. Thus, this region may be a hot spot for recombination in the PWK mouse strain.

Cross #2 Results: [(C57BL/6J-*gm* x SPRET)F_1 X C57BL/6J-*gm*] = ~300 progeny

- *gm* is localized to a 0.9cM interval.
- *gm* is flanked proximally by *D14Mit63* and distally by *D14Mit5* & *65*.
- *D14Mit83, 84, 122, 215,* & *261* cosegregate with *gm* in 300 meioses.
- This cross appears to have undergone a distance compression (recombination suppression) and polymorphism increase. Many more markers are polymorphic in this cross than in cross #1, however, most are not separable by genetic mapping. This region may be a recombination cold spot in the SPRET mouse strain.

The two *gm* crosses mentioned above have failed to determine an accurate genetic location of *gm*. The lack of polymorphic markers in the PWK cross has prohibited the mapping of many markers, although the ones that have been polymorphic were informative in localizing *gm* either proximal or distal to the marker. The SPRET cross has provided many polymorphic markers. However, the lack of recombination between B6 & SPRET in the *gm* region is such that the markers all segregate together, and thus *gm* can not be localized relative to many of them. With this starting point, further genetic mapping and positional cloning techniques were undertaken to clone the *gunmetal* gene, as described in the following chapters.

CHAPTER 2
IDENTIFICATION OF NOVEL SIMPLE SEQUENCE LENGTH POLYMORPHISMS (SSLP) IN MOUSE BY INTERSPERSED REPETITIVE ELEMENT (IRE)-PCR

Introduction

To address the need for additional markers in the *gm* region, the method described in this chapter was developed and employed.[22] Positional cloning is an important method of disease gene identification in human and in model organisms such as mouse. This approach is based on gene identification from contiguous genomic clones spanning a genetically defined interval that contains a locus of interest.[23] A prerequisite of positional cloning is the creation of dense genetic and physical maps for the chromosomal region of interest. One of the most informative and widely-used type of genetic markers is the simple sequence length polymorphism (SSLP), also known as polymorphic microsatellite markers.[24] Such markers are made up of serial di-, tri-, tetra- or penta-nucleotide repeats, where the number of repeat units differs among individuals/strains within a species. Microsatellite markers are easily amplified by PCR and exhibit strain-specific polymorphisms upon observation on agarose or polyacrylamide gels. However, for most regions of the mouse genome, current SSLP density is insufficient for positional cloning approaches.

Conventional approaches to the identification of novel SSLPs from a genomic region, such as subcloning genomic regions and hybridization with repeat probes, are cumbersome.

Interspersed repetitive element (IRE)-PCR is a very useful method for identification of novel human or mouse sequence tagged sites (STSs) from contigs of genomic clones or somatic cell hybrids.[25-28] This technique achieves selective amplification of mammalian DNA from an admixture with yeast or prokaryotic DNA, without prior knowledge of the mammalian DNA sequence, by PCR using outwardly oriented primers that are specific for mammalian high copy number repetitive elements. For example, IRE-PCR of mouse genomic DNA may be accomplished using primers designed from the 5' and 3' ends of the B1 repetitive element DNA sequence, which belongs to the short interspersed element (SINE) family (Figure 2.1). The 130-150 bp B1 repetitive element, which is believed to be homologous to the human 'Alu' repeat, is present at a copy number of 130,000-180,000 per haploid mouse genome,[29] and frequently is located at intron-exon junctions within genes.[30]

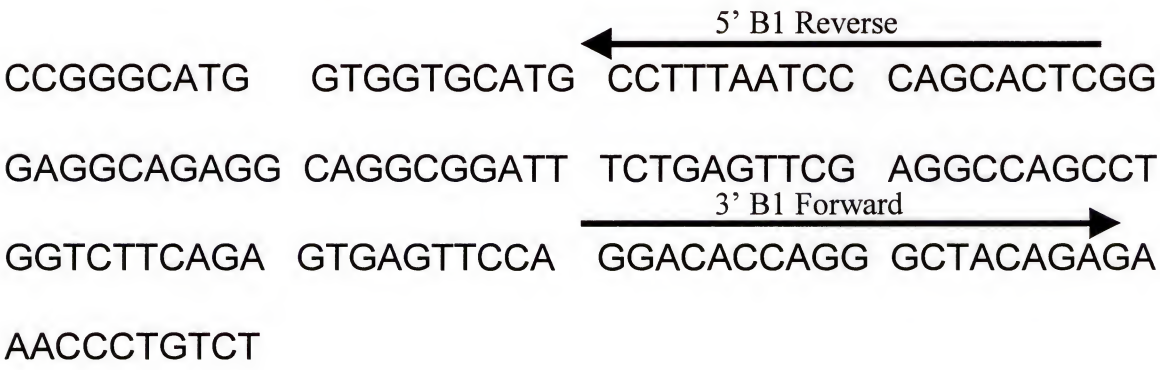

Figure 2.1: Consensus sequence of the B1 repetitive element.[28] Primers used for the IRE-PCR are identified above the sequence with arrows.[27]

Materials and Methods

As described below, B1-B1 IRE-PCR (Figure 2.2) was exploited to identify novel polymorphic SSLPs from genomic clones containing mouse DNA. Genomic DNA from 4 different mouse chromosome 14 YAC clones (63B10, 148G4, 183B10, and 200A8) was isolated as part of a positional cloning effort (see Chapter 4) and used as the template for three separate IRE-PCR reactions.

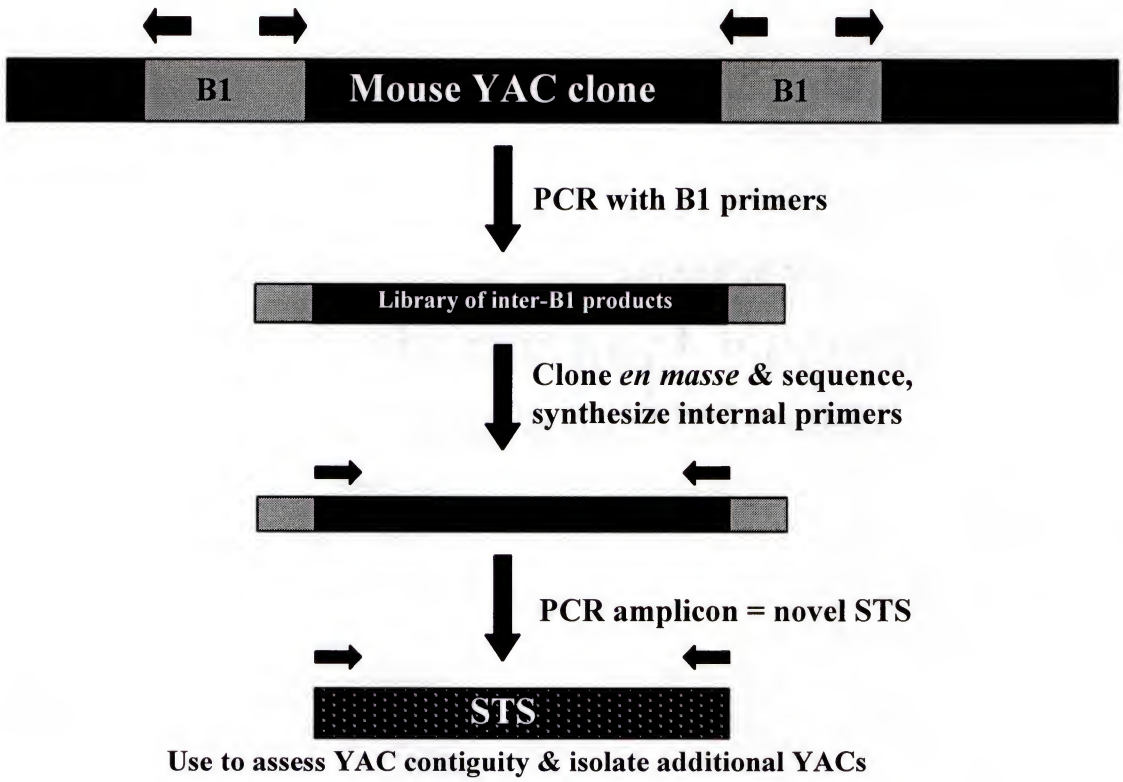

Figure 2.2: Flow diagram of the IRE-PCR method.[22]

The DNA was amplified with a 5' B1 reverse primer alone, a 3' B1 forward primer alone, and a combination of 5' B1 reverse and 3' B1 forward primers together (Figure 2.1).[22] The PCR master mixes contained (for one 50 μl reaction): 50 ng of yeast genomic DNA (containing a YAC), 1 μM of each primer, 200 μM of each dNTP, 1x PCR buffer, and 2.5 units of *Taq* DNA polymerase (Boehringer Mannheim, Indianapolis, IN). The PCR amplifications were performed in a PTC-100 Programmable Thermal Controller (MJ Research, Watertown, MA). The conditions involved an initial 2 min denaturation at 95°C; then 33 cycles of 95°C for 20 sec, 55°C for 20 sec, and 72°C for 90 sec; and finally a 10 min polishing extension at 72°C. Upon amplification, an aliquot of each reaction was electrophoresed on a 2% agarose gel to ensure amplicon (PCR product) complexity of at least 10 readily identified bands. Using these conditions, amplicons varied in size between 200 bp and 2500 bp. Following purification of the remaining 45 μl of PCR reaction (Wizard kit, Promega, Madison, WI), the three IRE-PCR products from each YAC were admixed and 6 μl of each pool was cloned *en masse* (TA cloning kit, Invitrogen, San Diego, CA). Twenty-two recombinant colonies were picked, grown in liquid culture, plasmid DNA was isolated (Wizard Plus Miniprep, Promega), and inserts were end-sequenced with M13 vector primers using an ABI PRISM 310 Genetic Analyzer and appropriate cycle sequencing reagents (Perkin-Elmer, Foster City, CA).

Results and Discussion

End sequence was obtained from 19 clones, 15 of which contained simple sequence repeats (at least two clones containing simple sequence repeats were identified in each of the four chromosome 14 YACs). Specific primers were designed from unique sequences flanking the repeat units in six clones to permit specific amplification of these loci. All gave a single amplicon of expected size upon PCR using the YAC clone from which they had been derived. Testing DNA from several mouse strains revealed that all six STSs were polymorphic (Table 2.1).[22] Genetic mapping in an intersubspecific backcross revealed that each of these polymorphisms mapped to the same region of mouse chromosome 14 as the YACs (see Chapter 4). Prior to these experiments the four chromosome 14 YACs were known to contain only three SSLPs. The experiments described here have identified six novel SSLPs within three of the four YACs. Table 2.1 lists the specific primer sequence for each marker along with the approximate amplicon size for each mouse strain tested.

In order to determine the general applicability of this method across the mouse genome, four YACs from another mouse autosome, chromosome 13, were used as template for B1-B1 IRE PCR. Of the 20 resultant amplicons that were cloned and end-sequenced, 14 contained simple sequence repeats, one of which was tested and found to be polymorphic (Table 2.1). The latter did map back to the region of mouse chromosome 13 from which the YAC clones were

derived (data not shown). These experiments showed that B1-B1 IRE-PCR is an efficient method for identification of novel mouse SSLPs. It should be noted that some of the remaining IRE-PCR products that may not contain simple sequence length polymorphisms are still useful as novel STSs.

Table 2.1: Novel simple sequence length polymorphisms.[22]

Novel SSLP	Primer Sequence (5' to 3')	Repeat Type	Product Size (bp)			
			B6	CAST	PWK	DBA
D14Sfk1	Forward = GAGAGAAACCCTGTCTCAAAC Reverse = CCATGGTGTATGTCACTTCAG	CAAA, AGGG, & AG	239	225*	240*	240*
D14Sfk2	Forward = CAGGACAGCCAGGGTTATAC Reverse = CTCAAGAAGATGCCGAATAG	CCAAA	224	250*	225*	ND
D14Sfk3	Forward = GGACAGCCAGGGTTATACTG Reverse = ATGTTATGTCACAGAATCAGC	CCAAA	141	160*	140*	150*
D14Sfk4	Forward = TTGGCCATGCAGGCAAACAC Reverse = CCTAATGTCTCCATTAACAAA	GT & GA	255	255*	270*	ND
D14Sfk5	Forward = GCAAGCCTTTAGAACAAGGC Reverse = GGAACTCTCAGGAAAAGTTAC	CA	109	135*	110*	125*
D14Sfk6	Forward = CTTGGGTAGGTCTTTCTTGC Reverse = ATGTTATGTAGTAATCAGTGGC	GAAA, GGAA, & GGAT	185	185*	190*	ND
D13Sfk1	Forward = CTCGAAAAGCCAAAAGAAG Reverse = GGCTTTAGATATGCAGTGAG	GAA	148	170*	160*	140*

The first column lists the novel polymorphic markers identified with the described method. The second column lists the forward and reverse primers (5' → 3') used to amplify each polymorphic marker. The third column lists the di-, tri-, tetra-, or penta-nucleotide repeats found in each polymorphic marker. The last column lists the product sizes (in base pairs) of each polymorphic marker when using B6 (C57BL/6JEi), CAST (CAST/Ei), PWK (wild-derived *Mus musculus musculus*), and DBA (DBA/2J) mouse strain DNA as template. B6 product sizes were based upon DNA sequence analysis. *CAST, PWK, and DBA product sizes are estimated, based on agarose gel electrophoresis analysis. ND = not determined. MGD accession number **J: 46919.**

The apparent association of SSLPs with B1 repetitive elements was unanticipated. However, the finding that sequences rich in A_mC_n blocks tend to flank B1 repetitive elements[30] may provide an explanation of the effectiveness of B1-B1 PCR in identifying novel SSLPs. The method developed here is a promising, simple approach to assist with the generation of dense genetic and physical maps surrounding mouse loci of interest, such as disease loci. The considerable homology shared between the mouse B1 repetitive element and the human 'Alu' repeat raises the possibility that this method may also be applicable to identifying human SSLPs. The SSLPs identified in the *gm* region were extremely valuable in narrowing the genetic region, to allow establishment of a better physical map and subsequent positional cloning, as described in the next few chapters.

CHAPTER 3
ISOLATION OF MOUSE TRANSCRIBED SEQUENCES FROM YAC/BAC CLONES USING AN INTERSPERSED REPETITIVE ELEMENT (IRE)-PCR BASED DIRECT cDNA SELECTION APPROACH

Introduction

Once the *gm* physical contig was constructed and narrowed using the additional markers identified with the method described in chapter 2, the isolation and identification of transcribed sequences within the contig, as part of positional cloning, became possible.

One of the most rate-limiting steps in positional cloning has been the identification of transcribed sequences (candidate disease genes) within a defined genomic region that contains the locus of interest. Both direct cDNA selection[31] and exon trapping[32] are well-characterized methods that have been used in numerous positional cloning efforts to identify candidate disease genes from genomic clones that lie within the region of interest. Of these methods, direct cDNA selection is more widely applicable because of its large target size, sensitive nature, tissue specificity, and relative speed.[27,33] In this technique, biotin-labeled, denatured genomic target (YAC etc.) DNA is hybridized in solution to tissue-specific denatured cDNA to which PCR-amplifiable tags have been added. The annealed complexes are captured and washed, and bound cDNAs

are eluted and PCR-amplified. Following two such rounds of selection, an ~10,000 fold enrichment is acquired.[31] Since its initial description in 1991, this method has undergone a number of modifications and improvements.[31,34] A significant limitation, however, has been the requirement of uncontaminated genomic template DNA to prevent false positives. Typically, YAC DNA is purified by preparative pulse field gel electrophoresis (PFGE). However, inadequate resolution of YAC and host yeast chromosomes frequently results in gross contamination of direct selection clones with yeast rRNA clones. Biotin labeling of the template, required for fluid-phase selection with magnetic separation, may also be inefficient, and requires radioactive monitoring during nick translation.

To circumvent the above limitations, we investigated the usefulness of interspersed repetitive element (IRE)-PCR for the generation of genomic template DNA (see Figure 2.2).[35] As previously described (Chapter 2),[22] IRE-PCR is a very useful method for selective amplification of mammalian DNA from an admixture with yeast or prokaryotic DNA, without prior knowledge of the mammalian DNA sequence, by PCR using outwardly oriented primers specific for mammalian high copy number repetitive elements such as B1 and B2. The 130-150 bp mouse B1 repetitive element, which is believed to be homologous to the human 'Alu' repeat, is present at a copy number of 130,000–180,000 per haploid mouse genome,[29] and frequently is located at intron-exon junctions within genes.[30]

As described below, IRE-PCR with biotin-labeled repetitive element primers (B1-B1, B2-B2, B1-B2) was exploited as a one-step method for isolating and biotin labeling selected mouse genomic template for direct cDNA selection (MGD # J:51591).[35] This novel approach amplifies and biotinylates genomic DNA flanking B1 and B2 repetitive elements from YAC/BAC clones. The amplified products are then used as the template in the previously described direct cDNA selection technique.[34]

Materials and Methods

Genomic DNA from four different mouse chromosome 14 YAC clones was isolated as part of a positional cloning effort (see Chapter 4)[36] and used as template for ten separate biotinylated IRE-PCR reactions. The DNA was amplified with all possible combinations of 5' B1 reverse ($^{5'}$CCGAGTGCTG-GGATTAAAGG$^{3'}$), 3' B1 forward ($^{5'}$GGACACCAGGGCTACAGAGA$^{3'}$), 5' B2 reverse ($^{5'}$ACTGAGCCATCTCTCCAG$^{3'}$), and 3' B2 forward ($^{5'}$ACATGGTGGC-TCACAACC$^{3'}$) repetitive element primers as previously described (Chapter 2),[22] with the exception that these primers were biotinylated at the 5' end (Gibco BRL, Gaithersburg, MD). Using reagents and conditions previously described (Chapter 2),[22] biotinylated amplicons varied in size from 200 to 2500 bp. Following purification (Qiagen, PCR purification kit, Santa Clarita, CA), the products from each YAC were pooled and ~ 100 ng was used as the target genomic template for direct cDNA selection (Figure 3.1).

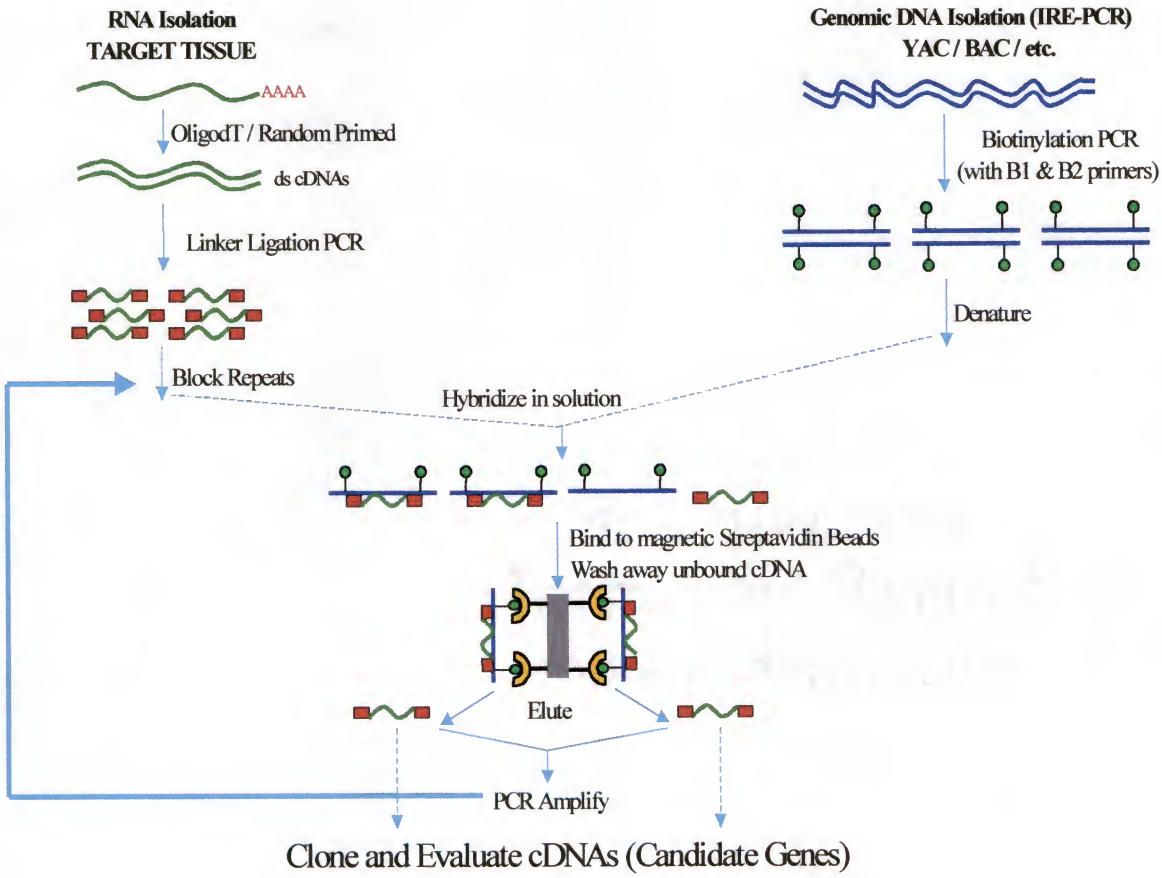

Figure 3.1. Schematic representation of the IRE-PCR modified direct cDNA selection procedure.[34,35] Top left depicts construction of the linkered cDNAs. Top right depicts generation of the target genomic DNA using IRE-PCR with biotinylated B1 and B2 primers. Bottom portion depicts the binding and elution of the selected cDNA fragments.

Random and oligodT primed mouse bone marrow cDNAs were generated using 1-5 μg of poly A^+ RNA (Gibco BRL, SuperScript choice system). Adhering to Del Mastro and Lovett's protocol,[34] ~3 μg of random primed and oligodT primed cDNAs underwent linker ligation (separately) to create two linkered cDNA sets. Linker primers were used to PCR amplify both sets of linkered cDNAs in 100 μl reactions. The amplified products were then purified and eluted (Qiagen), yielding 7.5 μg of amplified random linkered cDNA and 7.7 μg of amplified oligodT linkered cDNA.

Direct cDNA selection was then carried out as described by Del Mastro and Lovett.[34] Briefly, to block the cDNA against yeast DNA and rRNA contaminants, 1 μg of each purified linkered cDNA (2 μg total) was mixed with 1 μg total yeast DNA and 2 μg Cot I (Gibco BRL). The mixture was vacuum dried to a 10 μl volume, overlaid with oil, denatured for 5 min @ 95°C, chilled 5 min on ice, mixed with 10 μl 2X-hybridization solution, and incubated over night @ 65°C. Approximately 100 ng (in 15 μl) of the biotinylated target IRE-PCR products (isolated above from YAC genomic DNA) was overlaid with oil, denatured for 5 min @ 95°C, and chilled for 5 min on ice. At room temperature, 15 μl of 2X-hybridization buffer was added along with the 20 μl of blocked cDNA. The reaction mixture (genomic target + cDNA) was then incubated for 48 hrs @ 65°C. Following the Dynabeads protocol (Dynal, Oslo, Norway), cDNA bound to the target DNA was eluted, neutralized, and purified (Figure 3.1). The yield of eluted cDNA was 4.5 μg. Using linker primers, 250 ng of the eluted cDNA was PCR

amplified in a 100 μl reaction, purified, and tested for quality (smear) on an agarose gel. A second round of direct selection enrichment was carried out as above using 2 μg of the first round PCR amplified cDNA products and additional biotinylated target IRE-PCR DNA. The resulting second round eluted cDNA products were PCR amplified with UDG linker primers (Gibco BRL), purified, and cloned using the UDG pAMP10 vector system (Gibco BRL). End sequence (using T7 primer) was obtained from 80 clones, 26 of which either matched known genes or were novel ESTs (Table 3.1, IRE-DS #1).[35] All products (from genes and ESTs) mapped back to the target YACs used in the selection process. Half of the clones consisted of rRNA.

Table 3.1. Isolated direct cDNA selection products (MGD # J:51591).[35]

SUMMARY DATA	IRE-DS #1	IRE-DS #2
Number of clones sequenced	80	80
rRNA	40	23
B1 & contaminants	14	9
Unique Chr 14 Genes	**Number of Clones**	
Cathepsin G (*Ctsg*)	5 (2)*	0
Rab geranylgeranyl transferase (*Rabggta*)	4 (2)*	15 (2)*
Ubiquitin-like protein (*Nedd8*)	2	21 (3)*
Leukotrine B4 receptor (*Ltb4r*)	0	1
Multispanning membrane protein (*Mmp*)	0	5 (2)*
Novel Mast cell protease (*Mcpt*)	1	0
Novel ESTs	14	6
( )* = number of different gene regions isolated		

To maximize target DNA amplification between repetitive elements and to reduce rRNA contamination, long-range PCR and additional rRNA blocking reagents were utilized in a second direct cDNA selection experiment. Long-range PCR with Klentaq (Clonetech, Advantage cDNA PCR kit, Palo Alto, CA) and a 3 min extension was carried out using the same target YAC genomic DNA, biotinylated B1 and B2 primers, and conditions as above. Biotinylated target IRE-PCR products ranged in size from 600 bp to 6 kb. As carried out above, the linkered cDNA sets were blocked for contaminants, however, mouse rDNA (gift from Dr. Anand Srivastava) was also included as a blocking reagent. The blocked cDNA and target long-range IRE-PCR DNA were mixed, eluted, and cloned as above, but employed a different Dynabead kit (Dynal, kilobase BINDER) to efficiently bind the beads to the larger DNA fragments. End sequence showed that 48/80 clones matched known genes or were novel ESTs (Table 3.1, IRE-DS #2).[35] All 48 clones mapped back to the original target YACs. As shown in Table 3.1, these minor adjustments to our first direct cDNA selection procedure produced 59% fewer contaminants (rRNA, B1) and two additional genes.

Results and Discussion

Advantages to this IRE-PCR modification of direct cDNA selection include: (1) Purification of YAC DNA by PFGE is not required since yeast and *E. coli* do not contain B1 or B2 repetitive elements; (2) Use of 5'-biotin labeled B1/B2 primers ensures efficient generation of biotinylated target PCR fragments;

(3) False positive clones (rRNA and B1 contaminants) are reduced in frequency; and (4) Isolation of novel STSs flanking B1/B2 sequences may be useful for saturating a genomic region with novel genetic markers (we have recently reported that these regions are enriched for polymorphic simple sequence repeats, Detter *et al.*, 1998). Since B1 and B2 repetitive elements are abundant within the mouse genome, this approach results in relatively little loss of template complexity and is widely applicable. Genomic coverage is optimized by long-range PCR techniques.

Using the above IRE-PCR based direct cDNA selection approach (Figure 3.1), we identified five known genes and twenty-one novel ESTs on four YAC clones that span the critical region of our mouse mutation (Table 3.1).[35] Since four of the five genes identified were represented among different direct selection clones, this indicates that B1 and/or B2 repetitive elements are often interspersed throughout a gene, further illustrating the utility of this method. This powerful method is useful not only for positional cloning projects, but also for rapidly generating regional transcription unit maps. It is feasible that this method would also be applicable to other mammalian species by utilizing other high-copy, species-specific repetitive elements. As seen in chapter 4, we recently determined that one of the genes isolated (*Rabggta*) was the causative gene in our mouse mutant.[36]

CHAPTER 4
RAB GERANYLGERANYL TRANSFERASE MUTATION IN THE *GUNMETAL* MOUSE CAUSES DEFICIENT RAB27a PRENYLATION AND PLATELET SYNTHESIS

Introduction

As described in the preceding chapters, my work to this point led to identification of more markers in the *gm* region, and isolation of candidate cDNAs from the critical region YAC and BAC clones.[22,35] These two accomplishments were important steps in this project as prerequisites for the final stages: further narrowing of the *gm* interval, and selection and analysis of candidate cDNAs, to accomplish the positional cloning of the *gm* gene.

This chapter first describes the refined genetic mapping with the new markers, which narrowed the interval from 1.5 cM to 0.64 cM, and further describes a contig of genomic clones fully spanning this region.[36] The additional mapping data were useful in excluding some of the candidate cDNAs described in chapter 3. Among those cDNAs still within the *gm* interval, a portion were excluded from *gm* candidacy based on tissue expression patterns. The Rab geranylgeranyl transferase, alpha subunit gene (*Rabggt*a) was the top remaining candidate based on the protein's known function, prenylation of Rab GTPases. Briefly, Rabggta is one subunit of the Rab GGTase heterodimer which functions

to attach 20-carbon geranylgeranyl groups to small Rab proteins (Rab GTPases) (see Figure 5.1,).[37,38] This modification enables the Rab GTPases to bind to their target membranes and carry out their prescribed vesicular trafficking functions (including membrane budding and fusion reactions).[38] It made intuitive sense that loss or alteration of the alpha subunit could lead to *gm* features, because we would expect this to cause a lack of prenylated Rab GTPases, which in turn would reduce the amount of membrane budding and fusion necessary to package α-granules, δ-granules, and/or platelets. Furthermore, the tissue distribution and levels of Rabggta expression is consistent with the tissues most affected in *gm* mice. Based on these factors, this gene was chosen for further analysis in *gm* mice, and this chapter describes the genetic and biochemical analyses that revealed *Rabggta* to be the *gm* gene.[36]

Materials and Methods

Mice and Genetic Mapping

Mice obtained from the Jackson Laboratory (Bar Harbor, ME) were bred at Roswell Park Cancer Institute through our major collaborator, Dr. Richard Swank. Backcross mice were phenotyped by coat color (as *gm/gm* [gray] or *gm/+* [black]) and DNAs were then shipped to Florida where I genotyped for SSLP and RFLP using standard techniques.[39] Linkage relationships were determined by segregation analysis, and best locus order was decided by minimizing crossovers and eliminating double crossovers.[21]

Physical Mapping

Mouse YAC and BAC clones were identified from libraries[28] (Research Genetics, Huntsville, AL) by PCR or hybridization with oligonucleotides corresponding to loci in the vicinity of *gm*. Clones were oriented by sequence tagged site (STS) content mapping[40] and sequencing clone insert ends. Additional markers (SSLPs and genes) were isolated from YAC and BAC clones by IRE-PCR (see Chapter 2)[22] and direct cDNA selection (see Chapter 3),[35] and then placed on the contig based their location relative to other markers (see Figure 4.3). BACs were initially isolated with the assistance of Dr. Swank's lab.

IRE-Direct cDNA Selection

(see Chapter 3)[35]

Full-length *Rabggta* cDNA Isolation

5'- and 3'-RACE were performed with mouse fetal brain cDNA (Clontech, Palo Alto, CA). 5'-RACE PCR used a *Rabggta*-specific reverse primer (5'-TTCACAC-GCAGACAGCTCTC-3') and universal forward primer, followed by a second, nested PCR reaction with the *Rabggta* primer (5'-CTCTAGCCTTTTGGCC-TCTG-3') and second universal primer. 3'-RACE was performed using a *Rabggta*-specific forward primer (5'-CCAGCAGTCTGCTGCACTTC-3') and universal reverse primer, and a second, nested PCR used the *Rabggta* primer (5'-GGAGCGTTTGGCTGAGATGC-3') and another universal reverse primer. Products were subcloned and sequenced, and the full-length *Rabggta* cDNA sequence was assembled.

RT-PCR Analysis

Total RNA was prepared from +/+, *gm*/+, and *gm*/*gm* tissues (bone marrow, kidney, and melanocyte) with equilibrated phenol/guanidine isothiocyanate (Gibco BRL, Gaithersburg, MD). Oligo(dT)-primed, first-strand cDNA was synthesized with SuperScript II reverse transcriptase (Gibco BRL). All *Rabggta* exons were amplified by PCR with gene specific primers. Aberrant *gm* splicing was identified with the *Rabggta* primers 5'-GTGCAAGGGTCCACGGGAC-3' (exon α) and 5'-TTCACACGCAGACAGCTCTC–3' (exon 4).

Western-blot Analysis

Platelet and bone marrow cells were isolated as described[41] except that platelets were washed twice with 0.38% sodium citrate in 0.85% NaCl and once with 1% ammonium oxalate to remove residual red blood cells. 20 μg protein from *gm/gm*, *gm/+*, and C57BL/6J-+/+ tissues in proteinase inhibitor cocktail were incubated in SDS/mercaptoethanol and electrophoresed on denaturing 10% polyacrylamide gels followed by Western blotting on nitrocellulose or PVDF membranes.[16] For subcellular fractionation studies, fresh platelets were sonicated and separated into membrane and soluble subcellular fractions by centrifugation as described;[16] samples were immediately separated on 12% polyacrylamide gels. Western blots were probed with specific antisera against Rabggta (C222)[37] or Rab27a[42], followed by peroxidase-labeled goat anti-rabbit IgG secondary antibody (Kirkegaard & Perry, Gaithersburg, MD), and visualized by incubation with ECL-Plus luminescent reagent (Amersham, Piscataway, NJ) and autoradiography.[43] Several exposures were taken and comparisons were made in the linear range. Blots were washed and re-probed with antibodies to mouse actin. Alternatively, Western blots were hybridized with [^{32}P-GTP] and washed, followed by autoradiography, to detect GTP-binding proteins.[16] Quantitations were obtained using a phosphorimager (Molecular Dynamics, Sunnyvale, CA). Western analysis was accomplished by Dr. Richard Swank's laboratory at Roswell Park Cancer Institute, Buffalo, NY.

Assay of Rab GGTransferase Activity

Frozen tissues (liver, kidney, or spleen) were thawed, immersed in 3 volumes of buffer (50 mM sodium HEPES (pH 7.2), 5 mM $MgCl_2$, 10 mM NaCl, 1 mM dithiothreitol, 0.5 mM phenylmethylsulfonyl fluoride, 5 μg/ml aprotinin, 5 μg/ml pepstatin, and 5 μg/ml leupeptin) and lysed by polytron homogenization. Lysates were spun at 100,000 x g for 1 hr, and pellets discarded. Frozen platelet samples (10^7-10^8 platelets/pellet) were lysed in buffer by repeated passages through a Hamilton syringe. Protein concentrations were determined using Coomassie Plus reagent (Pierce).

Rab GGTase activity[42] was determined by measuring [^{3}H] transfer from [^{3}H]geranylgeranyl pyrophosphate to Rab1a in 50 μl reactions containing 50 mM sodium HEPES (pH 7.2), 5 mM $MgCl_2$, 1 mM dithiothreitol, 4 μM [^{3}H]GGPP (2,365 dpm/pmol), 5 μM recombinant Rab1a, 2 μM recombinant REP-1, and indicated amounts of cytosolic protein from *gm/gm* or +/+ tissues. After incubation for 15 min at 37°C, ethanol/HCl-precipitable radioactivity was measured on glass fiber filters. Assays with concentrations of cytosolic proteins <10 μg were carried out in 25 μl reactions containing 1 μM [^{3}H]GGPP (47,300 dpm/pmol) and incubated for 30 min at 37°C. This transferase activity assay was carried out by Dr. Miguel Seabra's laboratory (collaborator and developer of method) at Imperial College, London, UK.

Assay of GGTase-I Activity

GGTase-I activity[42] was determined by Dr. Seabra's lab by measuring [^{3}H] transfer from [^{3}H]geranylgeranyl pyrophosphate to Rac1 in a 25 μl reaction containing 50 mM sodium HEPES (pH 7.2), 150 mM KCl, 5 mM $MgCl_2$, 1 mM dithiothreitol, 1.2 μM [^{3}H]GGPP (16,133 dpm/pmol), 200 μM Zwittergent 3-14, 25 μM recombinant Rac1, and the indicated amounts of cytosolic proteins. After incubation for 30 min at 37°C, ethanol/HCl precipitable radioactivity was measured as above.

Results and Discussion

Genotyping of 29 loci in 3 *gm* backcrosses representing 2462 meioses, narrowed the *gm* critical region to 0.64 cM between *D14Mit63* and *D14Mit122* (Figure 4.1 and 4.2, Mouse Genome Database [MGD] accession J:52722).[36] Three crosses were utilized to minimize the effect of a recombination cold spot observed in this region in the [(C57BL/6J-*gm* x SPRET)F_1 X C57BL/6J-*gm*] backcross. A consensus genetic map was constructed using the three backcrosses to allow for physical contig construction (Figure 4.3a). Genetic markers flanking and within the *gm* critical region were used to isolate contiguous yeast artificial chromosome (YAC) and bacterial artificial chromosome (BAC) clones encompassing *gm* (Figure 4.3b). To complete the cloning of this interval, novel, polymorphic simple sequence repeats (*D14Sfk1*, *D14Sfk3*, and *D14Sfk5*; Figure 4.3a, MGD J:46919) and sequence tagged sites (STS) were isolated from critical region YACs by interspersed repetitive element PCR (Chapter).[22] They were mapped (Figure 4.3a,b) and used to isolate additional YACs and BACs until a tiling path of clones covered the interval (Figure 4.3b). A family of serine protease genes (*Mcpt1, Mcpt2, Mcpt4, Mcpt5, Mcpt9, McptL*, *Ctla1, Ctla5, Ctla6, Ctla7*, and *Ctsg*) were mapped within the *gm* critical region but were excluded from *gm* candidacy by virtue of incompatible distribution of expression or absence of mutations (data not shown).

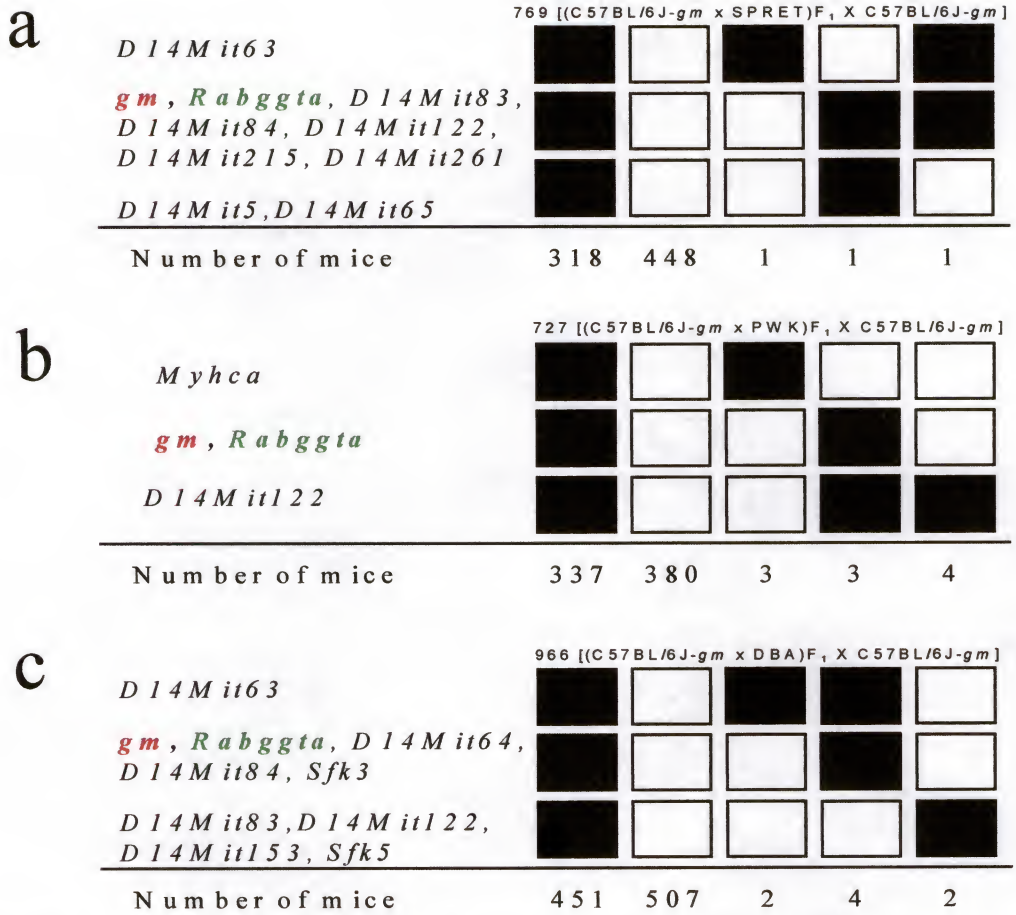

Figure 4.1: Mouse chromosome 14 haplotype analysis of three *gm* backcrosses. Black boxes represent homozygous gunmetal (*gm/gm*) mice based on coat color. White Boxes represent heterozygous gunmetal (SPRET, PWK, or DBA/*gm*) mice based on coat color. Genetic markers are to the left of the boxes and are arranged proximal (top) to distal (bottom) based on location. Numbers below the boxes represent the number of mice that coincide with the crossover pattern depicted by the box color and the genetic markers. **a,** Haplotype of 769 progeny produced by the [(C57BL/6J-*gm* x SPRET)F_1 X C57BL/6J-*gm*] backcross. Two mice have a crossover between *D14Mit63* and *gm* (etc.), and one mouse has a crossover between *gm* etc. and *D14Mit5*. No crossovers were observed between *D14Mit63* and *D14Mit5* in 766 progeny. **b,** Haplotype of 727 progeny produced by the [(C57BL/6J-*gm* x PWK)F_1 X C57BL/6J-*gm*] backcross. Six mice have a crossover between *Myhca* and *gm* (etc.), and four mice have a crossover between *gm* etc. and *D14Mit122*. No crossovers were observed between *Myhca* and *D14Mit122* in 717 progeny. **c,** Haplotype of 966 progeny produced by the [(C57BL/6J-*gm* x DBA)F_1 X C57BL/6J-*gm*] backcross. Two mice have a crossover between *D14Mit63* and *gm* (etc.), and six mice have a crossover between *gm* (etc.) and *D14Mit122* (etc.). No crossovers were observed between *D14Mit63* and *D14Mit122* (etc.) in 958 progeny. *gm* and *Rabggta* cosegregate with each other in all three crosses.

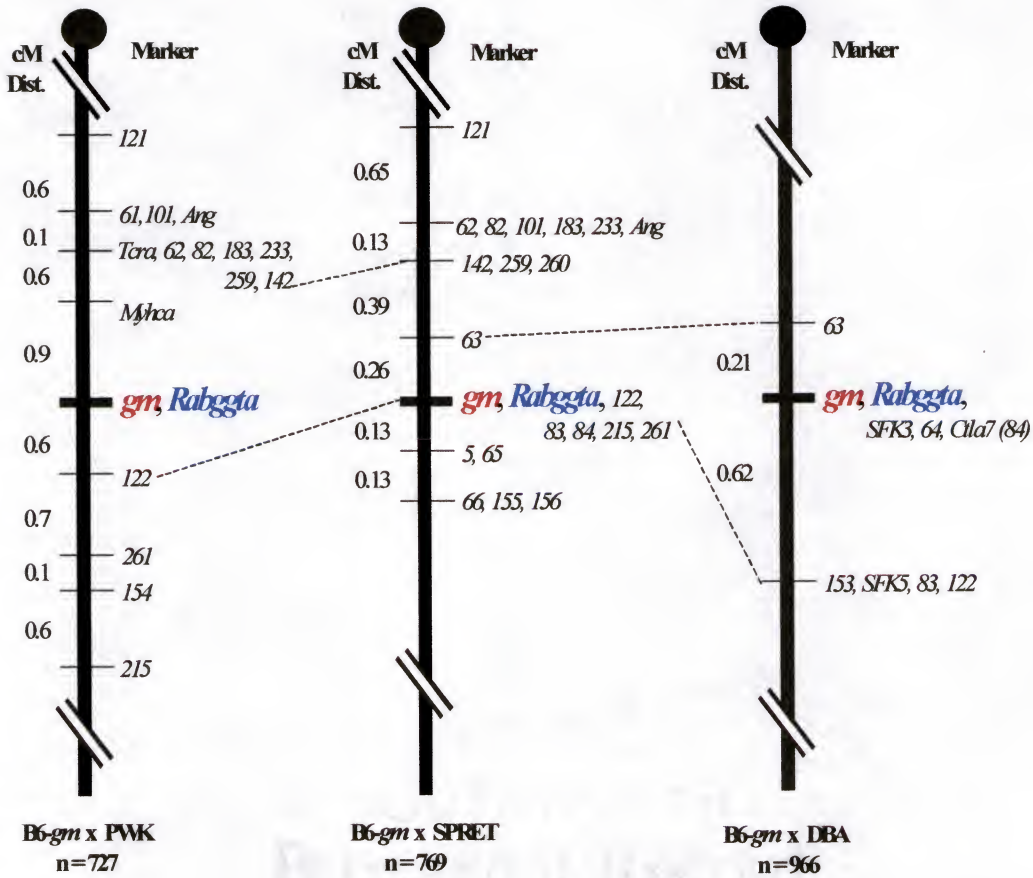

Figure 4.2: Mouse chromosome 14 genetic ideograms of the three *gm* backcrosses. Data in Figure 4.1 were used in the construction of these genetic maps. The genetic markers in the vicinity of *gm* are shown to the right of each stick figure. The markers are listed from proximal (top) to distal (bottom). The marker numbers correspond to *D14Mit* markers. The centiMorgan (cM) distances between the genetic markers are shown to the left of each ideogram. Dashed lines connect conserved genetic regions (same markers) between each backcross. *gm* and *Rabggta* cosegregate in all three crosses.

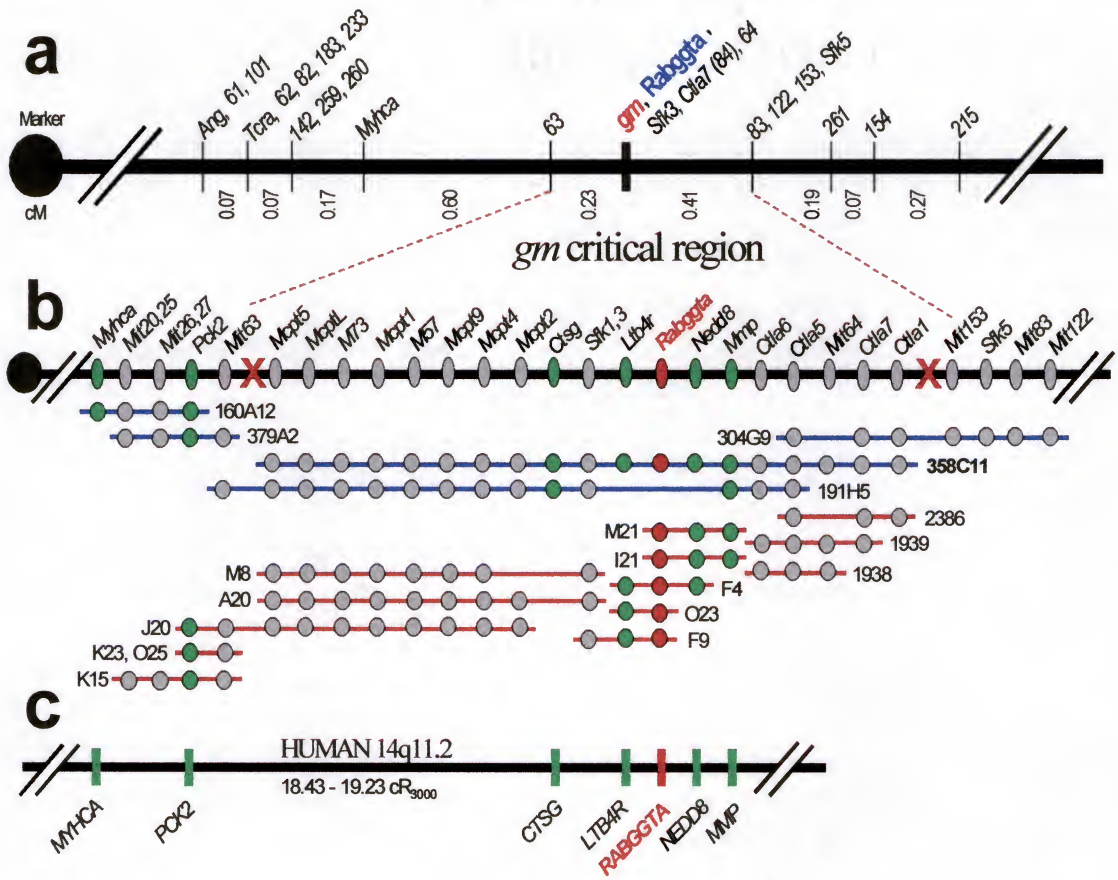

Figure 4.3: Genetic and physical maps of mouse Chr 14 in the vicinity of *gm*.[36] **a,** Consensus genetic map of the *gm* region on Chr 14 derived from 3 backcrosses (MGD J:52722). The circle (left) represents the centromere of Chr 14. Distances between markers are in cM below the chromosome, while loci are listed above. Numbered loci represent *D14Mit* microsatellite markers. Relative positions of loci were ascertained from 769 [(C57BL/6J-*gm* x SPRET)F_1 X C57BL/6J-*gm*], 727 [(C57BL/6J-*gm* x PWK)F_1 X C57BL/6J-*gm*], and 966 [(C57BL/6J-*gm* x DBA)F_1 X C57BL/6J-*gm*] backcross mice. No recombinations were found between *gm* and *Rabggta* in the 3 backcrosses (2462 meioses). Dotted lines link the consensus *gm* genetic map to the physical map using the loci *D14Mit63* and *D14Mit122*. **b,** Physical map of the *gm* non-recombinant interval (MGD J:52722). The *gm* critical region is delineated by chromosome crossovers (denoted by red X). *gm* was flanked by *D14Mit63* (proximal), and *D14Mit153* and *D14Mit122* (distal). YAC (blue lines) and BAC (red lines) clones are shown below the chromosome. Loci physically mapped within the YAC and BAC clones are indicated with colored circles. The location of *Rabggta* is indicated by red circles. Green circles indicate genes near *gm* that, **c,** also map to the homologous human chromosomal segment (14q11.2).[44]

Additional candidate genes were identified from pooled *gm* contig YACs and BACs using the modified direct cDNA selection method described in chapter 3 (MGD # J:51591).[35] Nineteen of 160 selected cDNA fragments were derived from a single candidate, *Rabggta*. Due to the logical connection between Rabggta function and the *gm* phenotype, this gene was the point of further focus as the leading candidate *gm* gene. *Rabggta*-derived STS were physically mapped on 6 *gm* critical region YAC / BAC clones (Figure 4.3b; MGD # J:52722).[36] A 2.2 kb *Mus spretus Bam*HI RFLP enabled genetic mapping of *Rabggta* in the [(C57BL/6J-*gm* x SPRET)F_1 X C57BL/6J-*gm*] backcross. *Rabggta* cosegregated with *gm* (Figure 4.3a, MGD # J:52722),[36] confirming derivation from the *gm* critical region.

Since mouse *Rabggta* was novel, overlapping cDNAs encompassing the full-length coding domain were isolated and sequenced (GenBank accession AF127656, see Appendix A). Mouse *Rabggta* cDNA shared 94% and 86% nucleotide identity to rat and human *Rabggta*, respectively. Rabggta is highly conserved, as evidenced by 33% predicted amino acid identity between mouse and the *S. cerevisiae* homologue, BET4 (see Appendix B).

Analysis of the genomic sequence of *Rabggta* revealed a single difference between C57BL/6J-+/+ and C57BL/6J-*gm/gm* DNA (GenBank AF127654 and AF127655, respectively): the normal 3' terminal nucleotide of intron α (guanine) was replaced with an adenine in *gm* DNA (Figure 4.4a & c).[36] This substitution created a novel *Tsp509I* restriction site that was used to show perfect cosegregation of *Rabggta* and *gm* in 2462 meioses (MDG # J:52722).[36]

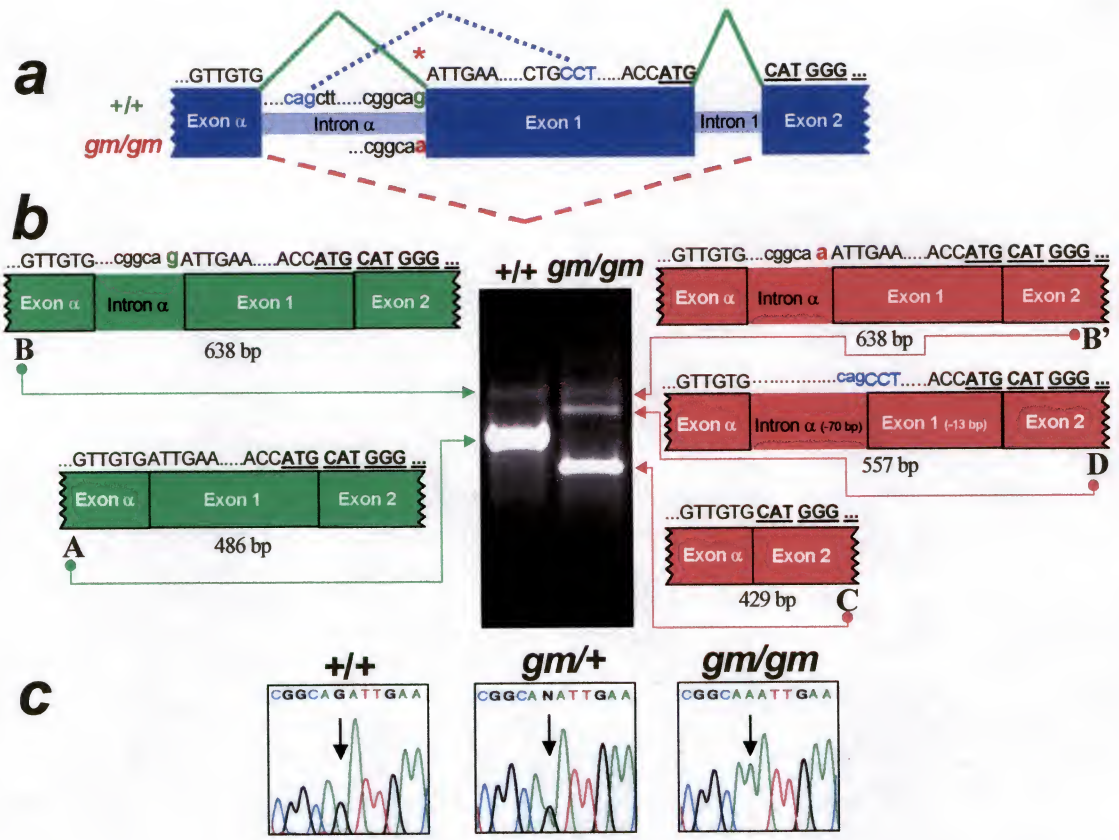

Figure 4.4: A splice acceptor mutation in *Rabggta* causes abnormal splicing.[36] **a,** genomic structure of the 5' end of mouse *Rabggta*. Exon α encodes 5' untranslated region. The initiation methionine (bold and underlined ATG) occurs at the 3' end of exon 1. *Rabggta* nucleotide sequence above is C57BL/6J-+/+, and below is C57BL/6J-*gm/gm*. The splice acceptor mutation is indicated by an asterisk. *Rabggta* exons connected by solid green lines represent normal splicing. Dashed red lines indicate exon 1 skipping in *gm/gm* RNA, and dotted blue lines indicate cryptic splice sites utilized by *gm/gm*. **b,** RT-PCR analysis of mRNA from wild-type (+/+) and mutant (*gm/gm*) bone marrow. The *Rabggta* oligonucleotides used amplified the region between exon α and exon 2. Normal bone marrow (+/+) shows the expected product of 486 bp (band A) (GenBank AF127656), and a product (638 bp) retaining intron α (band B) (GenBank AF127658). *gm/gm* bone marrow shows 3 bands: Band C (429 bp) (GenBank AF127657) represents exon 1 skipping; Band B' (638 bp) (GenBank AF127659) retains intron α; Band D (557 bp) (GenBank AF127662) represents utilization of cryptic splice sites in intron α and exon 1. **c,** Sequence analysis of *Rabggta* genomic DNA. Arrows indicate the splice acceptor mutation: +/+ =G, *gm/gm* = A, and *gm/+* exhibits both nucleotides (G and A).

Typically, substitution of the obligatory 3'-terminal guanine of the consensus splice acceptor site prevents normal mRNA splicing, and leads to intron retention, exon skipping, or cryptic splice site utilization.[45] Reverse transcription and PCR (RT-PCR) of the 5' end of *Rabggta* RNA (corresponding to exons α, 1, and 2) disclosed aberrantly spliced *Rabggta* transcripts in *gm* bone marrow (Figure 4.4b).[36] This tissue was used because the hematologic abnormalities of *gm* homozygotes are corrected by bone marrow transplantation.[17] In normal mice, RT-PCR of the 5' end of *Rabggta* RNA gave 2 products. The most abundant product was correctly spliced (exon α - exon 1 - exon 2; Figure 4.4b, +/+ product A). A second, minor product observed in normal mice retained intron α (exon α - intron α - exon 1 - exon 2; Figure 4.4b, +/+ product B) and may be immature, since it could only be marginally amplified by PCR from cytosolic or poly(A)+ RNA (data not shown).

In *gm* homozygotes, the major *Rabggta* transcript of +/+ bone marrow was not observed (product A, exon α - exon 1 - exon 2). Instead, the most abundant *gm Rabggta* product was novel and skipped exon 1 (Figure 4.4b, *gm/gm* product C; exon α - exon 2). Translation of this transcript was not anticipated to yield functional Rabggta protein since the initiation codon was lost, and no alternative, in-frame start codon was present (Figure 4.4b).

A second novel, but much less abundant, *gm Rabggta* product represented activation of a cryptic splice donor (within intron α, 84 bp downstream of the normal splice donor site) and acceptor sites (within exon 1, 13 bp downstream of the G→A substitution)[Figure 4.4b, *gm/gm* product D].

Importantly, utilization of these cryptic splice sites did not result in removal of the initiation codon. The existence of this less abundant transcript is a logical explanation for the presence of Rabggta in *gm* platelets, at a reduced level (see Figure 4.5).

Like +/+ bone marrow, a rare product that retained intron α was amplified from *gm/gm* RNA (exon α - intron α - exon 1 - exon 2; Figure 4.4b, product B'). This *gm/gm* transcript was also anticipated to be functional if polyadenylated, since the initiation codon of *Rabggta* is in exon 1, and therefore the retention of intron α (with the G→A substitution) should not disrupt translation. However, RT-PCR of cytosolic and poly(A)+ RNA suggested that it was an immature, non-polyadenylated RNA, like the corresponding transcript in +/+ bone marrow (data not shown).

These aberrant *Rabggta* splice variants were found in all tissues tested (*gm* bone marrow, melanocyte, and kidney; data not shown). As expected, both wild type and mutant products were observed in *gm/+* heterozygote RNA (data not shown). No other defect in splicing was observed; other regions of *Rabggta* mRNA produced RT-PCR products of expected size and sequence in +/+ and *gm/gm,* with the exception of a *Rabggta* isoform that retained intron 9, which was found in both +/+ and *gm/gm* mice (GenBank accession AF127660, AF127661). The reduction in *Rabggta* transcript levels in *gm* homozygotes was specific: Northern blots and quantitative PCR revealed no changes in the abundance of transcripts of other Rab geranylgeranyl transferase subunits (Rab geranylgeranyl transferase subunit β, Rab escort protein-1 or –2) in bone marrow or kidney of

gm homozygotes (data not shown). While absolute determination of the relative amounts of *Rabggta* mRNA isoforms in *gm/gm* and +/+ tissues was not possible (since the size differences could not be resolved on Northern blots), no difference was observed between *gm/gm* and +/+ tissues in the overall abundance of *Rabggta* mRNA (data not shown). Thus, the definition of a *gm*-specific mutation in *Rabggta* was extremely good evidence that this was the *gm* gene. However, biochemical analysis was also undertaken to show that this gene was responsible for the *gm* phenotype.

Western blots of *gm* platelets showed that the apparent consequence of aberrant *Rabggta* mRNA splicing was ~70% reduction in 60-kDa Rabggta protein in *gm* platelets (Figure 4.5).[36] It is likely that residual Rabggta protein in *gm/gm* platelets resulted from translation of the novel *gm Rabggta* transcript that utilized cryptic splice sites as described above (Figure 4.4b, *gm/gm* product D).

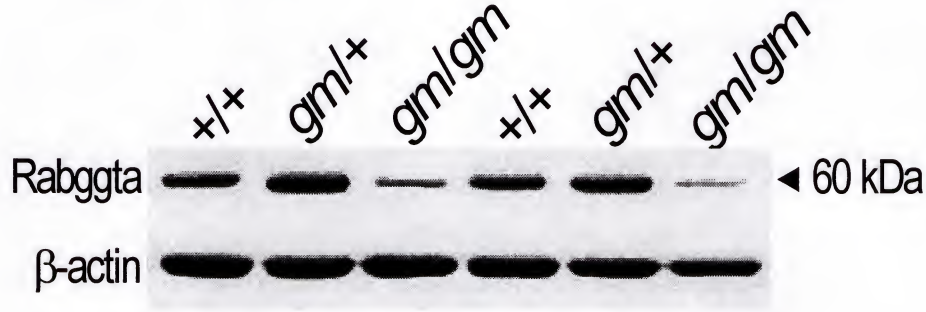

Figure 4.5: Decreased Rabggta protein levels in *gm/gm* mice.[36] Western blots of wild-type (+/+), heterozygous (*gm*/+), and mutant (*gm/gm*) platelets probed with Rabggta and β-actin antibodies show the 60-kDa Rabggta signal reduced ~70% in *gm/gm*, as compared to +/+. Platelets from two different mice from each genotype were analyzed for consistency.

Rabggta is part of Rab GGTase, a heterotrimeric protein comprising an α- and β-subunits, and Rab escort protein.[37,38] Rab GGTase activity in *gm/gm* and +/+ platelets was examined with an *in vitro* prenylation assay (Figure 4.6a).[36,42] *gm/gm* Rab GGTase activity was reduced ~70% compared with +/+ platelets (Figure 4.6a). Similar results were obtained with liver, kidney, and spleen extracts (Figure 4.6a and data not shown). It is unlikely that these results reflected prenylation of Rab1a by another enzyme (such as GGTase-I or farnesyl-transferase) since other enzymes do not prenylate this substrate.[46,47] In addition, the decrease in Rab GGTase activity matched the reduction in Rabggta protein. The *gm* reduction in Rab GGTase was specific: no difference was observed between *gm/gm* and +/+ in activity of GGTase-I, a related enzyme (Figure 4.6b). Thus, the *Rabggta* splicing defect in *gm* results in a biochemical defect: a partial decrease in Rabggta levels and Rab GGTase activity. It is interesting that while all *gm* tissues showed decreased Rab GTPase activity, phenotypic abnormalities were observed only in *gm* platelets and bone marrow. The sensitivity of platelets to Rab GGTase deficiency could be related to lack of a nucleus, and therefore decreased capacity for *de novo* protein synthesis; or, for reasons unknown, platelets require more Rab GGTase for normal function than other tissues.

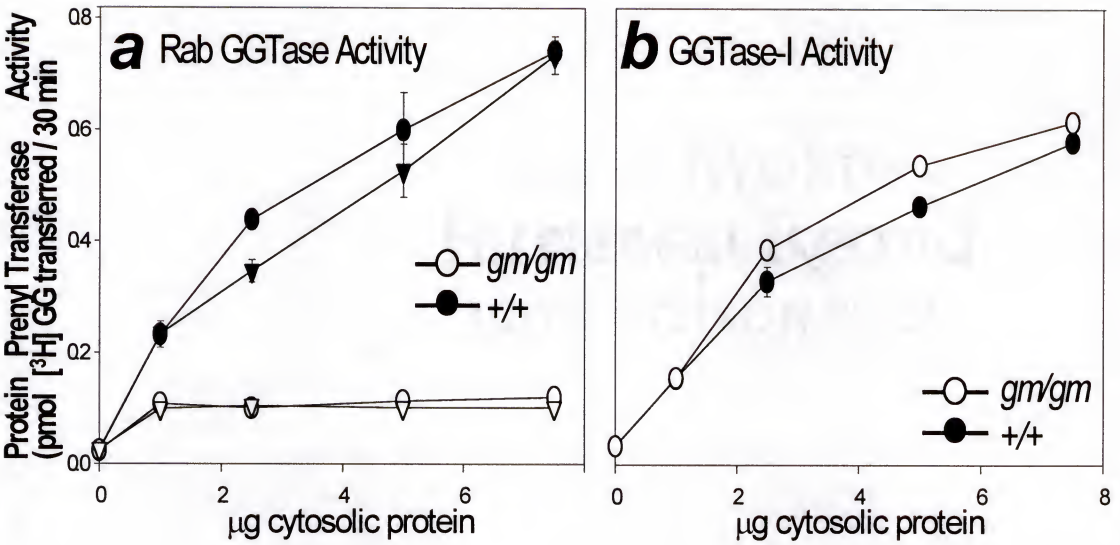

Figure 4.6: Decreased Rab GGTase activity in *gm/gm* mice.[36] **a,** Rab GGTase activity of *gm/gm* and +/+ platelets show ~70% reduction in *gm/gm* platelets. Duplicate cytosolic protein samples from pooled platelets (3 mice each) from gunmetal (*gm/gm*, open symbols) and C57BL/6J mice (+/+, solid symbols) were assayed for Rab GGTase activity by transfer of isoprenoid from ^{3}H-geranylgeranyl-pyrophosphate to Rab1a. **b,** GGTase-I activity of *gm/gm* and +/+ pooled platelets show no difference between the two. GGTase-I activity was determined by transfer of isoprenoid from ^{3}H-geranylgeranyl-pyrophosphate to Rac1. Each point represents an average of duplicate reactions. For Rab GGTase reactions, a control reaction incubated following addition of 50 mM EDTA was subtracted from all samples. For GGTase-I reactions, a control reaction incubated with buffer alone was subtracted.

A previous study showed that expression of some small GTP-binding proteins was abnormal in *gm* platelets.[16] Novel bands (~25- and ~28.5-kD) of unknown identity were observed on GTP overlays of Western blots of *gm* platelets (Figure1.3 and 4.8).[16,36] These were not expressed in *gm* liver, kidney, brain, spleen, macrophages or neutrophils.[16] Rab proteins represented

candidates for these abnormal GTP binding proteins. Specifically, Rab27a was an excellent candidate since it is abundant in melanocytes and platelets,[48] and is a substrate for prenylation by Rab GGTase.[42] Immunoblots disclosed a single Rab27a band of 27.5-kD in normal platelets and bone marrow, but an additional mobility-shifted (28.5-kD) isoform in *gm/gm* (Figure 4.7).[36] This isoform may represent unprenylated Rab27a since the electrophoretic mobility of unmodified Rab proteins is slower than prenylated forms in SDS-containing gels.[47,49] Not all small GTPases showed abnormal mobility; no differences between *gm/gm* and +/+ platelets were observed on immunoblots of Rab4 or Ras.[16]

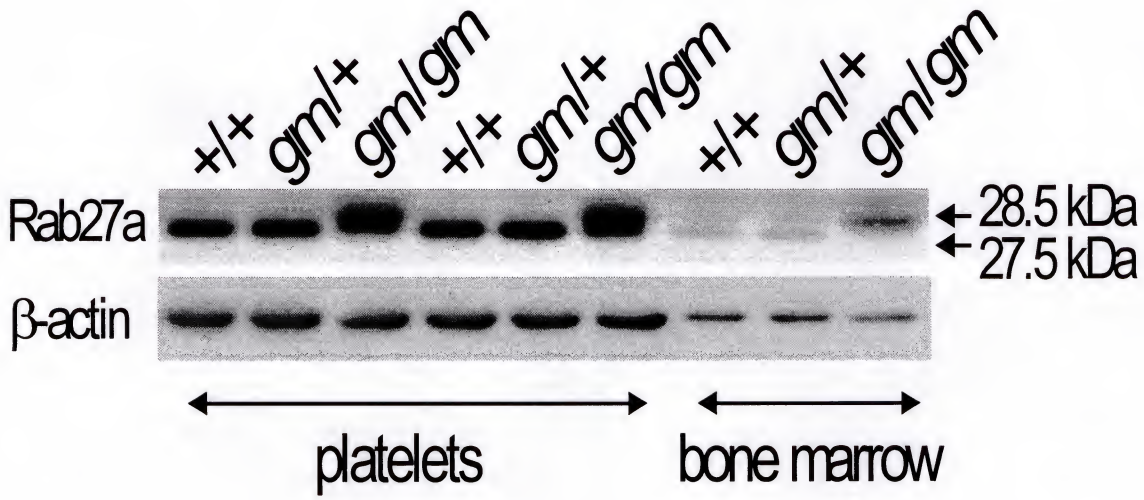

Figure 4.7: Deficient prenylation of Rab27a in *gm* platelets.[36] Western blots of wild-type (+/+), heterozygous (*gm*/+), and mutant (*gm*/*gm*) platelets and bone marrow incubated with Rab27a and β-actin antibodies identifies a shift in the *gm/gm* Rab27a lanes. This indicates hypoprenylation of Rab27a in *gm/gm* platelets. Platelets from two different mice from each genotype were analyzed for consistency.

Additional evidence for Rab27a hypoprenylation in *gm* was sought by examining *gm* platelets for membrane-associated Rab27a, since prenylation is required for membrane binding of Rab27a.[42] In analysis of subcellular protein fractions of platelets, Rab27a was mainly membrane-associated in normal platelets, but predominantly in the soluble fraction of *gm* platelets (Figure 4.8).[36] GTP overlay studies revealed that the distribution and mobility of the abnormal Rab27a band matched one of the previously described novel *gm* GTP binding proteins (Figure 1.3 and Figure 4.8).[16,36] Finally, immunohistochemical studies demonstrated punctate distribution of Rab27a in normal mouse platelets, consistent with localization on intracellular granules (Figure 4.9).[36] In contrast, *gm/gm* platelets exhibited more diffuse cytoplasmic Rab27a staining (Figure 4.9). This evidence suggests that Rab GGTase deficiency in *gm* platelets results in deficient Rab27a prenylation. Rab27a may be particularly affected in *gm* platelets because it has a low affinity for the REP2-containing Rab GGTase holoenzyme, and competes poorly with other prenylation substrates when Rab GGTase is limited.[42,48]

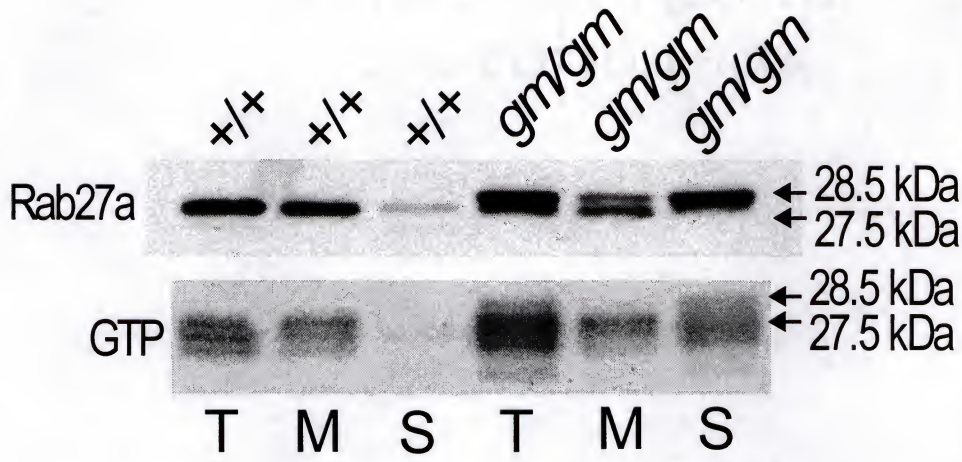

Figure 4.8: Deficient prenylation of Rab27a in *gm* platelets.[36] Western blots of subcellular fractions of wild-type (+/+) and mutant (*gm/gm*) platelets incubated with radiolabelled GTP or Rab27a antibodies. The subcellular fractions were total protein (T), membrane-associated protein (M), and soluble-fraction protein (S)].

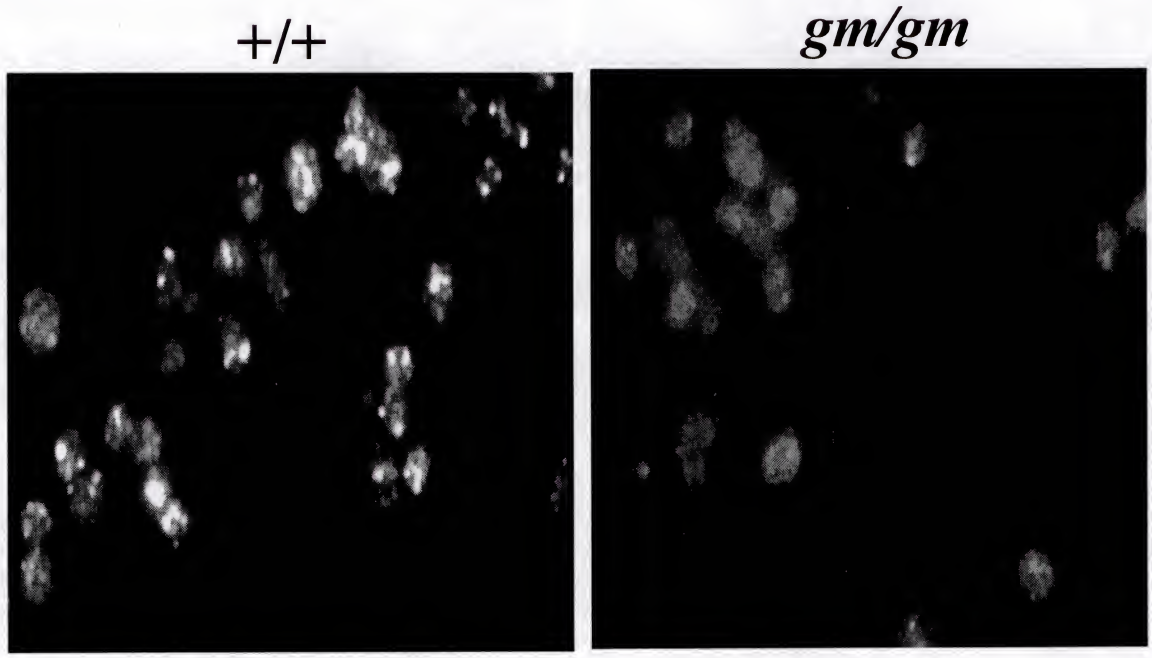

Figure 4.9: Differential intracellular localization of Rab27a in *gm* platelets compared to wild-type platelets.[36] Photomicrographs (500X) of +/+ and gm/gm platelets stained with Rab27a antibodies, and detected with an FITC-conjugated secondary antibody.

In this chapter I have described additional genetic and physical mapping to narrow the *gm* critical region and localize candidate genes relative to the *gm* loci. *Rabggta* was analyzed using genetic and biochemical assays, and was shown to be the causative gene of *gm*.[36] The facts that (1) this mutation allows leaky expression of *Rabggta*, and (2) only this one *gm* allele exists, suggests that homozygosity for completely null or inactivating *Rabggta* mutations might be lethal, and that the *gm* mouse produces enough Rabggta to allow viability. As discussed in the next chapter, the discovery that deficient Rab GGTase causes *gm* is of considerable significance for the study of platelet biology and other hematological conditions.

CHAPTER 5
CONCLUSIONS AND FUTURE DIRECTIONS

Despite numerous studies, relatively few molecular mechanisms or biochemical pathways regulating platelet production by megakaryocytes are known. This is a significant deficiency, since inherited and acquired platelet defects are not uncommon, and currently are treated with repeated platelet transfusions, which are very costly, may transmit infectious diseases, and may result in alloimmunization.[1,2] Identification of the molecular basis of Mendelian mouse models of platelet defects is an effective way to identify new and important pathways or factors involving platelet production from megakaryocytes. Therefore, the main objective of this dissertation work was to positionally clone and characterize the gene responsible for the mouse mutation *gunmetal*, in order to gain insights into the biological processes involving hematopoiesis. Five specific aims were established in order to achieve this objective:

1. Create a precise genetic map of mouse Chr 14 in the vicinity of *gm*.
2. Generate a physical map spanning the *gm* critical region.
3. Identify *gm* candidate genes based on location, function, & expression.
4. Screen *gm* candidate cDNAs for mutations.
5. Characterize the *gm* gene.

Below, the results for each specific aim are summarized, followed by final discussion and future directions.

Summary of Results

Create a Precise Genetic Map of Mouse Chr 14 in the Vicinity of *gm*

Three large *gm* backcrosses were constructed at the outset of this study, resulting in 2462 meioses. Twenty-nine polymorphic loci (some derived as described below) in the vicinity of *gm* were genotyped in the three backcrosses, thus narrowing the genetic interval to 0.64 cM between D14Mit63 and D14Mit122 (Figure 4.1, 4.2, and 4.3).[36] Three crosses were utilized to minimize the effects of observed polymorphism deficiencies and recombination cold spots.

Generate a Physical Map Spanning the *gm* Critical Region

A consensus genetic map of the *gm* critical region (Figure 4.3a)[36] was constructed from the backcross haplotype data (Figure 4.1). Genetic markers flanking and within the critical region were used to isolate contiguous YAC and BAC clones that encompass *gm*. To complete the cloning of the *gm* interval, novel polymorphic markers (SSLPs) and sequence tagged sites (STSs) were isolated using interspersed repetitive element (IRE)–PCR.[22]

IRE-PCR is a useful method for identification of novel human or mouse (STSs) from contigs of genomic clones (Figure 2.2). I used IRE-PCR with mouse B1 repetitive element primers to generate novel, PCR amplifiable, simple sequence length polymorphisms (SSLPs) from yeast artificial chromosome (YAC) clones containing regions of mouse chromosomes 14 (see Chapter 2, Table 2.1).[22] Twenty-nine out of 42 IRE-PCR clones contained multiple simple

sequence repeat units. PCR analysis with primers derived from unique sequences flanking the simple sequence repeat units in seven clones showed all to be polymorphic between various mouse strains. This novel approach to SSLP identification represents an efficient method for saturating a genomic interval with polymorphic genetic markers that may expedite (as it did in this project) the positional cloning of genes for traits and diseases.

Identify *gm* Candidate Genes Based on Location, Function, and Expression

After an overlapping physical contig of the *gm* critical region was constructed, I used direct cDNA selection to isolate and identify the genes that lie within the YAC and BAC clones of the contig (see Chapter 3).[35] Direct cDNA selection is a powerful method to identify candidate genes in a defined genomic region for positional cloning projects (Figure 3.1).

To overcome some of the limitations associated with isolating genomic template DNA for the direct selection procedure, I used IRE-PCR with biotinylated mouse B1 and B2 repetitive element primers to rapidly generate uncontaminated genomic template DNA from yeast artificial chromosome (YAC) clones (Figure 2.2 and 3.1). The resulting genomic template DNA was successfully used in the direct cDNA selection procedure described by Del Mastro and Lovett, resulting in identification of 5 known genes and 21 novel ESTs (Table 3.1).[35] One of the genes isolated (*Rabggta*) was later determined to be the causative gene of *gm* (see Chapter 4),[36] illustrating that our novel modification of the template preparation procedure for direct cDNA selection is

effective and may help expedite (as it did in this project) positional cloning of disease genes.

Screen *gm* Candidate cDNAs for Mutations

Several genes were screened for mutations in *gm* mice. Genomic sequence of Rab geranylgeranyl transferase, alpha-subunit (*Rabggta*) revealed a G → A point mutation in the last nucleotide of the 3' splice acceptor in intron α of *gm/gm* DNA when compared to +/+ DNA (see Chapter 4, Figure 4.4a,c).[36] As expected, RT-PCR of *gm/gm* mRNA showed that substitution of this obligatory 3'-terminal guanine of the spice acceptor site prevented normal splicing and led to exon 1 (contains start codon) skipping, intron α retention, and cryptic splice site utilization (Figure 4.4a,b).[36]

Due to the substantial loss of exon 1 and its start codon during *Rabggta* splicing in *gm/gm* mRNA, a significant deficiency (~70% loss) of Rabggta protein in *gm/gm* platelets was identified on Western blots (Figure 4.5).[36] This protein deficiency correlated with an ~70% reduction in Rab GGTase activity in *gm/gm* mice (Figure 4.6).[36] This excessive loss of Rab GGTase prenylation activity points to an explanation of the hematologic defects associated with the *gm* mouse and may lead to a better understanding of platelet biogenesis (see below).

Characterize the *gm* Gene

Rabggta not only fits as the causative gene of *gm* based on its genetic location and identified point mutation, but also based on its function. Rabggta is the alpha subunit of Rab GGTase. Rab GGTase is a heterodimer composed of tightly bound α- and β-subunits. It is inactive on its own, requiring another component, Rab Escort Protein (REP), for activation so that it may transfer two 20-carbon geranylgeranyl groups to Rab proteins (Rab GTPases) that bear double cysteine motifs at their COOH-terminus.[37,38] Rab GTPases then interact with membranes to regulate vesicular transport steps in endocytic and secretory pathways by acting as molecular switches oscillating between active GTP-bound and inactive GDP-bound states (Figure 5.1).

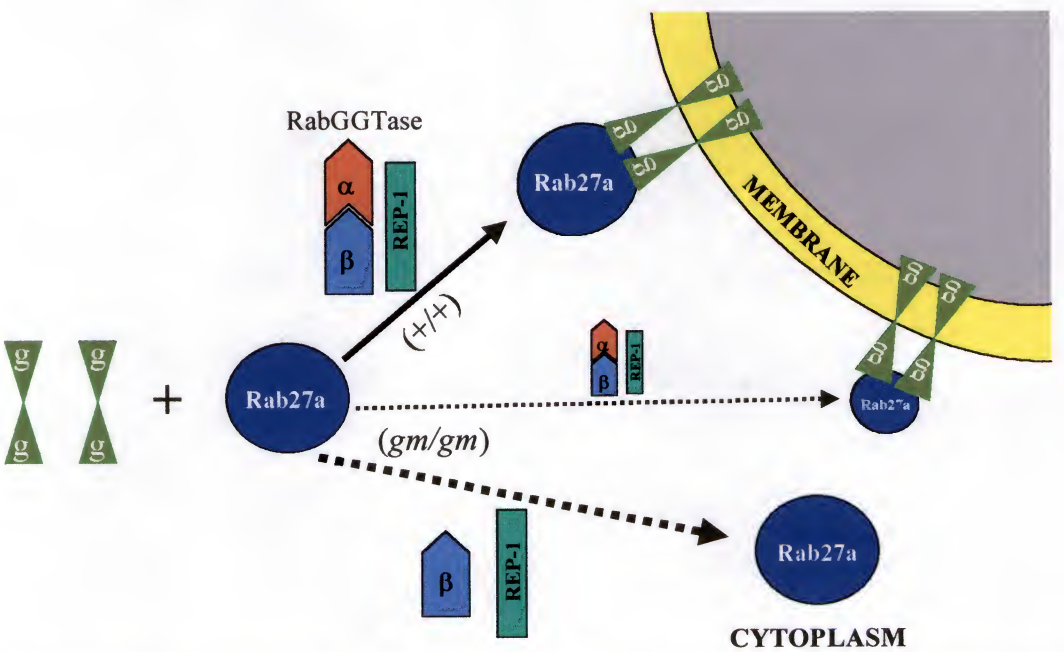

Figure 5.1: Diagrammatic representation of Rabggta's role in Rab prenylation and membrane attachment. (g) diamonds represent geranylgeranyl groups. Rab GGTase components are marked α, β, and REP-1. Top arrow (+/+) represents normal prenylation of Rab27a. Bottom two dashed arrows (*gm/gm*) represent reduced prenylation of Rab27a due to substantial deficiency of the Rabggta subunit.

Discussion

Most *gm* hematologic defects are explicable on the basis of impaired prenylation by Rab GGTase of substrates, such as Rab27a, ablating ability of these molecules to attach to membranes or regulate intracellular protein transport.[37,38,42,47,50] Rab proteins regulate intracellular vesicular transport by cycling between membrane-attached, GTP-bound and cytosolic, GDP-bound states.[38,50-53] Intracellular protein trafficking and vesicular transport are disordered in *gm*.[16,17] Proteins destined for delivery from the trans-Golgi network to α- and δ-granules are misrouted in *gm* platelets. As a result, *gm* platelet α-granules lack fibrinogen, von Willebrand factor (vWF) and platelet factor 4. vWF is secreted from *gm* megakaryocytes instead of being transported to α-granules.[16]

In addition to qualitative defects, the number of platelet α- and δ-granules and platelet synthesis is decreased in *gm* mice. Platelet synthesis is a complex process involving generation of large membrane complexes within megakaryocytes. The distribution of these membrane complexes is abnormal in *gm* megakaryocytes, and *gm* platelets are less common, larger and more heterogeneous in size than normal.[17] Importantly, the association of Rab GGTase with maintenance of normal platelet count and bleeding time implies that Rab GGTase is a therapeutic target for thrombocytosis and clotting disorders (such as myocardial infarction, stroke, or deep venous thrombosis). Compounds that selectively inhibit Rab GGTase have been developed[54] and

may be effective in these disorders if dosed to mimic *gm* by reducing platelet Rab GGTase activity by ~70%, and thus reducing overall clotting ability.

The molecular pathology of *gm* is strikingly similarity to the X-linked human disorder choroideremia (CHM). In both, mutated genes encoding subunits of Rab GGTase result in partial enzyme deficiency: *Rabggta* is mutated in *gm* and Rab geranylgeranyl transferase component A (REP1) is mutated in CHM.[55] Retention of partial Rab GGTase activity in *gm* probably reflects aberrant splicing that rescues certain *Rabggta* transcripts. In CHM, partial activity remains because of two redundant loci encode Component A of Rab GGTase (REP1 and REP2).[55,56] In both, a consequence of partial deficiency of Rab GGTase is decreased Rab27a prenylation, as evidenced by change in mobility and membrane-association.[42] The phenotype of *gm*, however, is rather different from CHM. Melanocytes are abnormal in both: coat color is diluted in *gm*, and retinal melanocytes are one of the first cell types affected in CHM.[42] However, albinism does not occur in CHM, nor have retinal abnormalities been observed in *gm*. Furthermore, CHM patients do not have prolonged bleeding time or platelet storage pool deficiency. These differences may reflect genetic complementation by REP2 in CHM platelets, but not *gm*. They may also reflect species differences, as suggested by phenotypic differences between CHM patients and *Chm* knockout mice.[57]

The human genetic disorders most similar to *gm* are gray platelet syndrome (GPS) and α/δ-SPD.[11,19,58] Like *gm*, GPS platelets are reduced in

number, increased in size, and lack α-granule proteins.[19,59] Mis-sorting and inappropriate secretion of α-granule proteins occurs in GPS, as in *gm*.[59] GPS patients, however, do not exhibit δ-granule storage pool deficiency or partial albinism. Like *gm*, α/δ-SPD is characterized by reduction in the number and contents of both α- and δ-granules.[11] Thus, *RABGGTA* is an excellent candidate gene for GPS and α/δ-SPD.

Future Directions

An important initiative is to identify the human disease(s) caused by mutations in *RABGGTA* (if any). This is often the ultimate goal of positionally cloning disease genes using mouse models. As mentioned above, GPS and α/δ-SPD are good human candidate diseases for *RABGGTA* mutations. Currently, patient blood samples are being sought for further analysis. If possible, both RNA and DNA will be isolated from the patient samples to be used in RT-PCR, Southern, Northern, and Western analyses. Additionally, direct sequence analysis will be done on both cDNA and DNA, including all intron/exon boundaries. Furthermore, some of the above mentioned experiments may or may not be necessary based on RT-PCR and other preliminary tests. Favorable outcome of these analyses may lead to better diagnostics, treatments, and understanding of hematological diseases. However, since it is likely that a *gm* null mutant would be lethal (based on my observation that the *gm* mutation is

leaky), I suspect this to be the case in humans as well, and thus it would not be unexpected to find the human mutation to be leaky as it is in *gm*.

If a mutation in *RABGGTA* is found to cause a hematological disease in humans (such as GPS or α/δ-SPD), it is plausible that gene therapy could be used for treatment. Experimental evidence that shows reciprocal bone marrow transplants between *gm/gm* and *gm/+* mice correct or cause the mutant phenotype[17] suggests that clinical abnormalities can be permanently corrected in patients with these conditions. Since patient age (these conditions are not immediately life threatening) and cDNA size (only 2.4 kb) do not appear to be limiting factors, it may be feasible to remove bone marrow (relatively easy to isolate and manipulate) from an affected human patient, transfect stem cells with a normal RABGGTA-producing construct into the bone marrow, and then transplant it back into the patient. Such a gene therapy approach could be tested and perfected using *gm* mice first.

Another future direction for the gunmetal project involves derivation of mouse transgenics to test whether re-introduction of *Rabggta* corrects the *gm* phenotype. At present, experiments to introduce cDNA and BAC transgenes of the normal *Rabggta* gene into *gm* mice are in their early stages. With the assistance of collaborators at Baylor College of Medicine, we will test whether the *gm* phenotype in recessive *gm* mice is corrected by creating heterozygous transgenics carrying a normal copy of *Rabggta*. Both cDNA and BAC strategies will be attempted, to overcome the limitations associated with each type of

transgene. Currently, a full-length (5'UTR through 3'UTR) *Rabggta* cDNA product has been cloned (using TOPO TA kit) and sequenced (no point mutations present). The next step will be to clone the *Rabggta* cDNA into a vector with a strong universal eukaryotic promoter (e.g. pCAGGS).[60] The construct will be sent to our collaborators at Baylor, who already have established the *gm* mouse colony on site. They will inject the cDNA construct into *gm/gm* fertilized oocytes, and then transfer these oocytes into the uterus of a pseudo-pregnant wild-type mouse. All progeny will be *gm* homozygotes at the original locus, but will not have the diluted coat color (or mutant platelet phenotype) if the transgene has incorporated correctly and is producing sufficient Rabggta. As a caveat, over-expression of the cDNA transgene could cause unanticipated problems in scoring phenotype. Thus, BAC transgenics may also be utilized to test the effects of the gene under its normal expression controls. BACs, however, are more difficult to manipulate due to their large size, and the possibility exists that they may not contain the complete promoter and/or enhancer systems necessary for normal expression. Considering all such factors, a transgenic result of an abnormal phenotype does not dis-prove that *Rabggta* is the *gm* gene. However, if it is successful, it would provide final proof that *Rabggta* is the gene that causes *gm*. Furthermore, even if complete phenotype correction is not seen, these experiments may still produce valuable information concerning protein over-expression or mis-expression.

Another future project related to this work concerns the beta subunit (Rabggtb) of the Rab GGTase heterodimer. At present, no human or mouse

diseases have been reportedly linked to mutations in *Rabggtb*. However, since two of the three components of the Rab GGTase heterodimer have been found to be perturbed, causing disease (REP-1 causes *CHM* and Rabggta causes *gm*),[36,42] it is perfectly reasonable that the third component (Rabggtb) also causes disease when mutated.

Initial mapping has localized *RABGGTB* to human[38] 1p31 and mouse[61] Chr 3, cM 76.5. Interestingly, also localized to mouse Chr 3 (cM 74.8) is the semidominant mouse mutation varitint-waddler (*Va*).[62] Heterozygous mutants exhibit reduced fertility, deafness, and minor head-tossing and hyperactivity. Homozygous mutants exhibit severe infertility, high mortality, and intense behavioral abnormalities. Furthermore, like *gm* homozygotes, the *Va* mutants express coat color dilutions as well. Heterozygous coats are variegated with patches of normal-colored, diluted, and white fur. Homozygotes show more dilute colors; their coats are white, except for small patches of unaltered color near the ears and base of the tail.[62] The pathological changes in heterozygotes include degeneration of the organ of Corti, stria vascularis, spiral ganglion, saccular macula, cristae ampullares, and vestibular ganglion. In homozygotes, the degenerative changes are more severe and also include the utricular macula.[63]

Although the characteristics associated with *Va* do not parallel those of *gm*, neither are *gm* characteristics similar to features of CHM. Furthermore, Rab3a (which is known to be modified by Rab GGTase) has been associated with neurological development since it controls exocytosis in neuroendocrine and

neuronal cells.[64] For these reasons, it is plausible that a mutation in *Rabggta* could cause *Va*. A positional candidate approach could be used to investigate this disease in the mouse model, as well as other possible candidate diseases that may exist in both humans and mouse. Currently, only *Va* homozygous DNA is available from Jackson Labs, as the mouse (C57BL/6J-*Va*) exists only as a frozen embryo. At a cost of $500, Jackson labs could provide these mice within 3 months. During the 3-month waiting period, preliminary Southern analysis could be done using *Va/Va*, *Va*/+, and +/+ DNA, hybridized to a *Rabggtb* probe. When RNA became available from the mice, RT-PCR and Northern analysis could be done to further assay for mutations. Additionally, since *Rabggtb* is relatively small (1011 bp coding region), sequence analysis could be achieved quickly and at a fairly low cost. Should these analyses fail to identify a mutation, a more conventional positional cloning approach could be undertaken if so desired.

APPENDIX A
MOUSE *RABGGTA* SEQUENCES SUBMITTED TO GENBANK

GenBank Submission AF127654

```
Submission 1 of a set of 9 submission(s).

Comment: Intron alpha (bp 125-276), Intron 1 (bp 334-471), bp 276 contains (g)
which is mutated to (a) in the gunmetal mutant strain (sequence will be submitted
for this in the next submission).

LOCUS       XXXXX        2685 bp    DNA             ROD       11-FEB-1999
DEFINITION  Mus musculus RAB geranylgeranyl transferase, alpha subunit
            (Rabggta) DNA, first 2 introns and all cds exons.
ACCESSION   ;
KEYWORDS    .
SOURCE      house mouse.
  ORGANISM  Mus musculus
            Eukaryota; Metazoa; Chordata; Vertebrata; Mammalia; Eutheria;
            Rodentia; Sciurognathi; Muridae; Murinae; Mus.
REFERENCE   1  (bases 1 to 2685)
  AUTHORS   Detter,J.C., Novack,E.K., Seabra,M, Swank,R.T. and Kingsmore,S.F.
  TITLE     Abnormal Protein Prenylation Leads to Hypopigmentation/Platelet
            Storage Pool Deficiency in the Gunmetal Mouse Mutant
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 2685)
  AUTHORS   Detter,J.C.
  TITLE     Direct Submission
  JOURNAL   Submitted (11-FEB-1999) Molecular Genetics & Microbiology, and
            Medicine, University of Florida, 1600 SW Archer Rd, Gainesville,FL
            32610, USA
FEATURES             Location/Qualifiers
     source          1..2685
                     /organism="Mus musculus"
                     /strain="C57BL/6J"
                     /db_xref="taxon:10090"
                     /chromosome="14"
                     /map="between Mit63 and Mit122"
BASE COUNT      544 a    771 c    758 g    612 t
ORIGIN
        1 gtgcaagggt ccacgggacg gacggacggg cgggcgggca ctgctctcta tatgcgcggc
       61 gtgtgcctcg gccctgacgg gtggggcttc ggaaagatcc gtggggacgg aggccgtggt
      121 tgtggtgagc gcttcctcct gacctcacag tgccagccgc cggccccctt ttaactcacg
      181 gggatcctta tcctgggcct gctggcagct tccgggaaac acctgggggg cggggccgtg
      241 tgggcggggt tctgtgattg acatctcttt cggcagattg aacgggctgc ctccggaccc
      301 ctcccctatc gccaacgctg agccgaaacc atggtgagaa ccctgagctc agccccttтc
      361 ccctgcatgc ccatgccctc atcccacgtg gctatctcgg ccgggcttct gcaagactgc
      421 ccttgcgagg caaccggcgt tcatattatc ctatcctcat atccctggta gcatgggcgc
      481 ctgaaggtga agacgtccga agagcaggca gaggccaaaa ggctagagcg ggaacagaag
      541 ctaaagctct accagtcagc cactcaagct gtcttccaga agcgggaggc aggcgagctg
      601 gatgaatccg tcctagaact gacaagccag attctgggag ccaaccctga ttttgccacc
      661 ctctggaact gtcgcagaga agtgctccag cagctagaaa cccagaagtc ccctgaggag
      721 ttggctgctc ttgtgaaggc agaactaggc ttccttgaga gctgtctgcg tgtgaaccct
```

```
     781 aagtcctatg gcacttggca ccaccgctgc tggctgctga gtcgcctgcc tgagcccaac
     841 tgggcccggg agctggagct gtgcgctcgc ttcctcgagg ccgatgagcg gaactttcat
     901 tgctgggact atcggcgatt tgtagctgca caggctgctg tcgcgcctgc agaggaacta
     961 gccttcactg acagcctcat cacccggaac ttctccaact attcttcctg gcattatcgc
    1021 tcctgcctct tgccccagct gcacccccag ccagactctg gcccccaggg acggctccct
    1081 gaaaatgtac tgctgagaga gctggagttg gtgcagaatg ccttctttac tgaccccaat
    1141 gatcagagtg cctggttcta tcaccgctgg ctcctggggc gggcggagcc ccacgatgtt
    1201 ctgtgctgcc tgcatgtgag ccgggaagag gcctgtctgt cagtctgctt ttctcgtccc
    1261 ctgatagtgg gctccaagat ggggaccttg ctactcacgg ttgacgaggc acctttgagt
    1321 gtggaatgga ggactccaga tggcaggaac cggcccagcc atgtctggct ctgtgacctg
    1381 cctgctgcct ctctcaatga ccacttgccc cagcacacgt ttcgtgtcat ctggacagga
    1441 agcgataccc agaaggagtg tgtgctttta aaaggtcacc aggagtgctg gtgccgagac
    1501 tcagccactg atgaacagtt gttcaggtgt gagctgtccg tggagaagtc cacagtgcta
    1561 cagtccgagc ttgagtcctg taaggagctg caggaactgg agcctgagaa taaatggtgc
    1621 ctgctgacca tcatcctcct gatgagggca ctggaccccc tcctctacga gaaagaaaca
    1681 ctggagtact tcagtaccct caaagctgtg gacccgatga gagcagccta cttggacgac
    1741 ctgcgcagca agttcttggt ggaaaacagt gttctcaaga tggagtatgc tgacgtgcgt
    1801 gtgctgcacc tggctcacaa ggatctcaca gtgctctgcc atttggaaca actgctcttg
    1861 gtcactcatc ttgacctgtc ccataatcgt ctccgagcct tgcccccagc cctggctgct
    1921 ctgcgctgtc tcgaggtgtt gcaggccagt gataatgtcc tggagaactt ggacggcgtg
    1981 gctaaccttc cccggctgcg ggagctgtta ctatgtaata accgcctcca gcagtctgct
    2041 gcacttcaga ctcttgcctc ctgccccagg ctagtcttcc tcaacctgca gggcaactcg
    2101 ctgtgccaag aagagggcat ccgggagcgt ttggctgaga tgctaccgtc cgtcagcagc
    2161 atcctcacct aggaggcccc atctcaccct cacccttaa cttattggga ctgaataaag
    2221 actggagagg cccctctagg ctgccaagct accattactg ctgccctgac aacacttcct
    2281 ccattaccac cactgtgcat tttggaaaaa ggaagggagg ggctctctgg ggtatggggc
    2341 cactgtcctg tgattctgtc taccaagacc ctcagtgttt atggctggtc aggaccaggg
    2401 ctgggtctga gttcaccatc agttttgggc tatcgtggtg cctgagcttg aagctgggat
    2461 aggaattagg atcaggttgt ggtttggact gctcatcaga agagctgtct cggggctgga
    2521 gagatggctc agcggttaag agcgctgact gttcttctga aagtcctgag ttcaaatccc
    2581 agcaaccaca tggtggctca taaccatctg taataagatc tgacaccctc ttctggtgtc
    2641 tgaagacagc tacagtgtac ttacatataa taataaataa atctt
//
```

GenBank Submission AF127655

Submission 2 of a set of 9 submission(s).

Comment: bp 276 contains the pt mutation (a). This mutation is in the first intron at the 3' acceptor. My previously submitted sequence contains the correct nucleotide (g).

```
LOCUS       XXXXX        2685 bp    DNA             ROD       11-FEB-1999
DEFINITION  Mus musculus (with gunmetal mutation) RAB geranylgeranyl
            transferase, alpha subunit (Rabggta), first two introns and all cds
            exons.
ACCESSION   ;
KEYWORDS    .
SOURCE      house mouse.
  ORGANISM  Mus musculus
            Eukaryota; Metazoa; Chordata; Vertebrata; Mammalia; Eutheria;
            Rodentia; Sciurognathi; Muridae; Murinae; Mus.
REFERENCE   1  (bases 1 to 2685)
  AUTHORS   Detter,J.C., Novack,E.K., Seabra,M, Swank,R.T. and Kingsmore,S.F.
  TITLE     Abnormal Protein Prenylation Leads to Hypopigmentation/Platelet
            Storage Pool Deficiency in the Gunmetal Mouse Mutant
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 2685)
  AUTHORS   Detter,J.C.
  TITLE     Direct Submission
  JOURNAL   Submitted (11-FEB-1999) Molecular Genetics & Microbiology, and
            Medicine, University of Florida, 1600 SW Archer RD, Gainesville,FL
            32610, USA
FEATURES             Location/Qualifiers
     source          1..2685
                     /organism="Mus musculus"
                     /strain="C57BL/6J-gm/gm"
                     /db_xref="taxon:10090"
                     /chromosome="14"
                     /map="between Mit63 and Mit122"
BASE COUNT      545 a    771 c    757 g    612 t
ORIGIN
        1 gtgcaagggt ccacgggacg gacggacggg cgggcgggca ctgctctcta tatgcgcggc
       61 gtgtgcctcg gccctgacgg gtggggcttc ggaaagatcc gtggggacgg aggccgtggt
      121 tgtggtgagc gcttcctcct gacctcacag tgccagccgc cggccccctt ttaactcacg
      181 gggatcctta tcctgggcct gctggcagct tccgggaaac acctgggggg cggggccgtg
      241 tgggcggggt tctgtgattg acatctcttt cggcaaattg aacgggctgc ctccggaccc
      301 ctcccctatc gccaacgctg agccgaaacc atggtgagaa ccctgagctc agccccttтc
      361 ccctgcatgc ccatgccctc atcccacgtg gctatctcgg ccgggcttct gcaagactgc
      421 ccttgcgagg caaccggcgt tcatattatc ctatcctcat atccctggta gcatgggcgc
      481 ctgaaggtga agacgtccga agagcaggca gaggccaaaa ggctagagcg ggaacagaag
      541 ctaaagctct accagtcagc cactcaagct gtcttccaga agcgggaggc aggcgagctg
      601 gatgaatccg tcctagaact gacaagccag attctgggag ccaaccctga ttttgccacc
      661 ctctggaact gtcgcagaga agtgctccag cagctagaaa cccagaagtc ccctgaggag
      721 ttggctgctc ttgtgaaggc agaactaggc ttccttgaga gctgtctgcg tgtgaaccct
      781 aagtcctatg gcacttggca ccaccgctgc tggctgctga gtcgcctgcc tgagcccaac
      841 tgggcccggg agctggagct gtgcgctcgc ttcctcgagg ccgatgagcg gaactttcat
      901 tgctgggact atcggcgatt tgtagctgca caggctgctg tcgcgcctgc agaggaacta
      961 gccttcactg acagcctcat cacccggaac ttctccaact attcttcctg gcattatcgc
     1021 tcctgcctct tgccccagct gcacccccag ccagactctg gcccccaggg acggctccct
     1081 gaaaatgtac tgctgagaga gctggagttg gtgcagaatg ccttctttac tgaccccaat
     1141 gatcagagtg cctggttcta tcaccgctgg ctcctggggc gggcggagcc ccacgatgtt
     1201 ctgtgctgcc tgcatgtgag ccgggaagag gcctgtctgt cagtctgctt ttctcgtccc
     1261 ctgatagtgg gctccaagat ggggaccttg ctactcacgg ttgacgaggc acctttgagt
     1321 gtggaatgga ggactccaga tggcaggaac cggcccagcc atgtctggct ctgtgacctg
     1381 cctgctgcct ctctcaatga ccacttgccc cagcacacgt ttcgtgtcat ctggacagga
     1441 agcgataccc agaaggagtg tgtgctttta aaaggtcacc aggagtgctg gtgccgagac
     1501 tcagccactg atgaacagtt gttcaggtgt gagctgtccg tggagaagtc cacagtgcta
```

```
     1561 cagtccgagc ttgagtcctg taaggagctg caggaactgg agcctgagaa taaatggtgc
     1621 ctgctgacca tcatcctcct gatgagggca ctggaccccc tcctctacga gaaagaaaca
     1681 ctggagtact tcagtaccct caaagctgtg gacccgatga gagcagccta cttggacgac
     1741 ctgcgcagca agttcttggt ggaaaacagt gttctcaaga tggagtatgc tgacgtgcgt
     1801 gtgctgcacc tggctcacaa ggatctcaca gtgctctgcc atttggaaca actgctcttg
     1861 gtcactcatc ttgacctgtc ccataatcgt ctccgagcct tgcccccagc cctggctgct
     1921 ctgcgctgtc tcgaggtgtt gcaggccagt gataatgtcc tggagaactt ggacggcgtg
     1981 gctaaccttc cccggctgcg ggagctgtta ctatgtaata accgcctcca gcagtctgct
     2041 gcacttcaga ctcttgcctc ctgccccagg ctagtcttcc tcaacctgca gggcaactcg
     2101 ctgtgccaag aagagggcat ccgggagcgt ttggctgaga tgctaccgtc cgtcagcagc
     2161 atcctcacct aggaggcccc atctcaccct caccctttaa cttattggga ctgaataaag
     2221 actggagagg cccctctagg ctgccaagct accattactg ctgccctgac aacacttcct
     2281 ccattaccac cactgtgcat tttggaaaaa ggaagggagg ggctctctgg ggtatggggc
     2341 cactgtcctg tgattctgtc taccaagacc ctcagtgttt atggctggtc aggaccaggg
     2401 ctgggtctga gttcaccatc agttttgggc tatcgtggtg cctgagcttg aagctgggat
     2461 aggaattagg atcaggttgt ggtttggact gctcatcaga agagctgtct cggggctgga
     2521 gagatggctc agcggttaag agcgctgact gttcttctga aagtcctgag ttcaaatccc
     2581 agcaaccaca tggtggctca taaccatctg taataagatc tgacaccctc ttctggtgtc
     2641 tgaagacagc tacagtgtac ttacatataa taataaataa atctt
//
```

GenBank Submission AF127656

```
Submission 3 of a set of 9 submission(s).

Comment: Please keep all data CONFIDENTIAL until in press.  Thanks!

LOCUS       XXXXX        2395 bp    mRNA            ROD       11-FEB-1999
DEFINITION  Mus musculus RAB geranylgeranyl transferase, alpha subunit
            (Rabggta)mRNA, complete cds.
ACCESSION   ;
KEYWORDS    .
SOURCE      house mouse.
  ORGANISM  Mus musculus
            Eukaryota; Metazoa; Chordata; Vertebrata; Mammalia; Eutheria;
            Rodentia; Sciurognathi; Muridae; Murinae; Mus.
REFERENCE   1  (bases 1 to 2395)
  AUTHORS   Detter,J.C., Novack,E.K., Seabra,M, Swank,R.T. and Kingsmore,S.F.
  TITLE     Abnormal Protein Prenylation Leads to Hypopigmentation/Platelet
            Storage Pool Deficiency in the Gunmetal Mouse Mutant
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 2395)
  AUTHORS   Detter,J.C.
  TITLE     Direct Submission
  JOURNAL   Submitted (11-FEB-1999) Molecular Genetics & Microbiology, and
            Medicine, University of Florida, 1600 SW Archer RD, Gainesville,FL
            32610, USA
FEATURES             Location/Qualifiers
     source          1..2395
                     /organism="Mus musculus"
                     /strain="C57BL/6J"
                     /db_xref="taxon:10090"
                     /chromosome="14"
                     /map="between Mit63 and Mit122"
     mRNA            join(1..124,125..181,182..292,293..419,420..605,606..809,
                     810..891,892..979,980..1078,1079..1185,1186..1239,
                     1240..1326,1327..1416,1417..1532,1533..1647,1648..1733,
                     1734..2395)
                     /gene="Rabggta"
     gene            1..2395
                     /gene="Rabggta"
     CDS             179..1882
                     /gene="Rabggta"
                     /codon_start=1
                     /product="RABGGTA"
                   /translation="MHGRLKVKTSEEQAEAKRLEREQKLKLYQSATQAVFQKREAGEL
                   DESVLELTSQILGANPDFATLWNCRREVLQQLETQKSPEELAALVKAELGFLESCLRV
                   NPKSYGTWHHRCWLLSRLPEPNWARELELCARFLEADERNFHCWDYRRFVAAQAAVAP
                   AEELAFTDSLITRNFSNYSSWHYRSCLLPQLHPQPDSGPQGRLPENVLLRELELVQNA
                   FFTDPNDQSAWFYHRWLLGRAEPHDVLCCLHVSREEACLSVCFSRPLIVGSKMGTLLL
                   TVDEAPLSVEWRTPDGRNRPSHVWLCDLPAASLNDHLPQHTFRVIWTGSDTQKECVLL
                   KGHQECWCRDSATDEQLFRCELSVEKSTVLQSELESCKELQELEPENKWCLLTIILLM
                   RALDPLLYEKETLEYFSTLKAVDPMRAAYLDDLRSKFLVENSVLKMEYADVRVLHLAH
                   KDLTVLCHLEQLLLVTHLDLSHNRLRALPPALAALRCLEVLQASDNVLENLDGVANLP
                   RLRELLLCNNRLQQSAALQTLASCPRLVFLNLQGNSLCQEEGIRERLAEMLPSVSSIL
                   T"
BASE COUNT      502 a    672 c    678 g    543 t
ORIGIN
        1 gtgcaagggt ccacgggacg gacggacggg cgggcgggca ctgctctcta tatgcgcggc
       61 gtgtgcctcg gccctgacgg gtggggcttc ggaaagatcc gtggggacgg aggccgtggt
      121 tgtgattgaa cgggctgcct ccggacccct cccctatcgc caacgctgag ccgaaaccat
      181 gcatgggcgc ctgaaggtga agacgtccga agagcaggca gaggccaaaa ggctagagcg
      241 ggaacagaag ctaaagctct accagtcagc cactcaagct gtcttccaga agcgggaggc
      301 aggcgagctg gatgaatccg tcctagaact gacaagccag attctgggag ccaaccctga
      361 ttttgccacc ctctggaact gtcgcagaga agtgctccag cagctagaaa cccagaagtc
```

```
 421 ccctgaggag ttggctgctc ttgtgaaggc agaactaggc ttccttgaga gctgtctgcg
 481 tgtgaaccct aagtcctatg gcacttggca ccaccgctgc tggctgctga gtcgcctgcc
 541 tgagcccaac tgggcccggg agctggagct gtgcgctcgc ttcctcgagg ccgatgagcg
 601 gaactttcat tgctgggact atcggcgatt tgtagctgca caggctgctg tcgcgcctgc
 661 agaggaacta gccttcactg acagcctcat cacccggaac ttctccaact attcttcctg
 721 gcattatcgc tcctgcctct tgccccagct gcacccccag ccagactctg gcccccaggg
 781 acggctccct gaaaatgtac tgctgagaga gctggagttg gtgcagaatg ccttctttac
 841 tgaccccaat gatcagagtg cctggttcta tcaccgctgg ctcctggggc gggcggagcc
 901 ccacgatgtt ctgtgctgcc tgcatgtgag ccgggaagag gcctgtctgt cagtctgctt
 961 ttctcgtccc ctgatagtgg gctccaagat ggggaccttg ctactcacgg ttgacgaggc
1021 acctttgagt gtggaatgga ggactccaga tggcaggaac cggcccagcc atgtctggct
1081 ctgtgacctg cctgctgcct ctctcaatga ccacttgccc cagcacacgt ttcgtgtcat
1141 ctggacagga agcgataccc agaaggagtg tgtgctttta aaaggtcacc aggagtgctg
1201 gtgccgagac tcagccactg atgaacagtt gttcaggtgt gagctgtccg tggagaagtc
1261 cacagtgcta cagtccgagc ttgagtcctg taaggagctg caggaactgg agcctgagaa
1321 taaatggtgc ctgctgacca tcatcctcct gatgagggca ctggaccccc tcctctacga
1381 gaaagaaaca ctggagtact tcagtaccct caaagctgtg gacccgatga gagcagccta
1441 cttggacgac ctgcgcagca agttcttggt ggaaaacagt gttctcaaga tggagtatgc
1501 tgacgtgcgt gtgctgcacc tggctcacaa ggatctcaca gtgctctgcc atttggaaca
1561 actgctcttg gtcactcatc ttgacctgtc ccataatcgt ctccgagcct tgcccccagc
1621 cctggctgct ctgcgctgtc tcgaggtgtt gcaggccagt gataatgtcc tggagaactt
1681 ggacggcgtg gctaaccttc cccggctgcg ggagctgtta ctatgtaata accgcctcca
1741 gcagtctgct gcacttcaga ctcttgcctc ctgccccagg ctagtcttcc tcaacctgca
1801 gggcaactcg ctgtgccaag aagagggcat ccgggagcgt ttggctgaga tgctaccgtc
1861 cgtcagcagc atcctcacct aggaggcccc atctcaccct cacccttaa cttattggga
1921 ctgaataaag actggagagg cccctctagg ctgccaagct accattactg ctgccctgac
1981 aacacttcct ccattaccac cactgtgcat tttggaaaaa ggaagggagg ggctctctgg
2041 ggtatggggc cactgtcctg tgattctgtc taccaagacc ctcagtgttt atggctggtc
2101 aggaccaggg ctgggtctga gttcaccatc agttttgggc tatcgtggtg cctgagcttg
2161 aagctgggat aggaattagg atcaggttgt ggtttggact gctcatcaga agagctgtct
2221 cggggctgga gagatggctc agcggttaag agcgctgact gttcttctga aagtcctgag
2281 ttcaaatccc agcaaccaca tggtggctca taaccatctg taataagatc tgacaccctc
2341 ttctggtgtc tgaagacagc tacagtgtac ttacatataa taataaataa atctt
//
```

GenBank Submission AF127657

Submission 4 of a set of 9 submission(s).

Comment: This cDNA sequence is the mutant form. A pt mutation in intron alpha causes a loss of exon 1, which contains the start codon.

LOCUS XXXXX 2338 bp mRNA ROD 11-FEB-1999
DEFINITION Mus musculus (with mutation) RAB geranylgeranyl transferase, alpha subunit (Rabggta)mRNA, complete cds (mutation in intron alpha causes loss of exon 1 which contains START).
ACCESSION ;
KEYWORDS .
SOURCE house mouse.
ORGANISM Mus musculus
Eukaryota; Metazoa; Chordata; Vertebrata; Mammalia; Eutheria; Rodentia; Sciurognathi; Muridae; Murinae; Mus.
REFERENCE 1 (bases 1 to 2338)
AUTHORS Detter,J.C., Novack,E.K., Seabra,M, Swank,R.T. and Kingsmore,S.F.
TITLE Abnormal Protein Prenylation Leads to Hypopigmentation/Platelet Storage Pool Deficiency in the Gunmetal Mouse Mutant
JOURNAL Unpublished
REFERENCE 2 (bases 1 to 2338)
AUTHORS Detter,J.C.
TITLE Direct Submission
JOURNAL Submitted (11-FEB-1999) Molecular Genetics & Microbiology, and Medicine, University of Florida, 1600 SW Archer RD, Gainesville,FL 32610, USA
FEATURES Location/Qualifiers
source 1..2338
/organism="Mus musculus"
/strain="C57BL/6J-gm/gm"
/db_xref="taxon:10090"
/chromosome="14"
/map="between Mit63 and Mit122"
BASE COUNT 490 a 649 c 665 g 534 t
ORIGIN

```
        1 gtgcaagggt ccacgggacg gacggacggg cgggcgggca ctgctctcta tatgcgcggc
       61 gtgtgcctcg gccctgacgg gtggggcttc ggaaagatcc gtggggacgg aggccgtggt
      121 tgtgcatggg cgcctgaagg tgaagacgtc cgaagagcag gcagaggcca aaaggctaga
      181 gcgggaacag aagctaaagc tctaccagtc agccactcaa gctgtcttcc agaagcggga
      241 ggcaggcgag ctggatgaat ccgtcctaga actgacaagc cagattctgg gagccaaccc
      301 tgattttgcc accctctgga actgtcgcag agaagtgctc cagcagctag aaacccagaa
      361 gtcccctgag gagttggctg ctcttgtgaa ggcagaacta ggcttccttg agagctgtct
      421 gcgtgtgaac cctaagtcct atggcacttg gcaccaccgc tgctggctgc tgagtcgcct
      481 gcctgagccc aactgggccc gggagctgga gctgtgcgct cgcttcctcg aggccgatga
      541 gcggaacttt cattgctggg actatcggcg atttgtagct gcacaggctg ctgtcgcgcc
      601 tgcagaggaa ctagccttca ctgacagcct catcacccgg aacttctcca actattcttc
      661 ctggcattat cgctcctgcc tcttgcccca gctgcacccc cagccagact ctggcccccа
      721 gggacggctc cctgaaaatg tactgctgag agagctggag ttggtgcaga atgccttctt
      781 tactgacccc aatgatcaga gtgcctggtt ctatcaccgc tggctcctgg ggcgggcgga
      841 gccccacgat gttctgtgct gcctgcatgt gagccgggaa gaggcctgtc tgtcagtctg
      901 cttttctcgt cccctgatag tgggctccaa gatggggacc ttgctactca cggttgacga
      961 ggcacctttg agtgtggaat ggaggactcc agatggcagg aaccggccca gccatgtctg
     1021 gctctgtgac ctgcctgctg cctctctcaa tgaccacttg ccccagcaca cgtttcgtgt
     1081 catctggaca ggaagcgata cccagaagga gtgtgtgctt ttaaaaggtc accaggagtg
     1141 ctggtgccga gactcagcca ctgatgaaca gttgttcagg tgtgagctgt ccgtggagaa
     1201 gtccacagtg ctacagtccg agcttgagtc ctgtaaggag ctgcaggaac tggagcctga
     1261 gaataaatgg tgcctgctga ccatcatcct cctgatgagg gcactggacc ccctcctcta
     1321 cgagaaagaa acactggagt acttcagtac cctcaaagct gtggacccga tgagagcagc
     1381 ctacttggac gacctgcgca gcaagttctt ggtggaaaac agtgttctca agatggagta
     1441 tgctgacgtg cgtgtgctgc acctggctca caaggatctc acagtgctct gccatttgga
     1501 acaactgctc ttggtcactc atcttgacct gtcccataat cgtctccgag ccttgccccc
     1561 agccctggct gctctgcgct gtctcgaggt gttgcaggcc agtgataatg tcctggagaa
```

```
     1621 cttggacggc gtggctaacc ttccccggct gcgggagctg ttactatgta ataaccgcct
     1681 ccagcagtct gctgcacttc agactcttgc ctcctgcccc aggctagtct tcctcaacct
     1741 gcagggcaac tcgctgtgcc aagaagaggg catccgggag cgtttggctg agatgctacc
     1801 gtccgtcagc agcatcctca cctaggaggc cccatctcac cctcaccctt taacttattg
     1861 ggactgaata aagactggag aggcccctct aggctgccaa gctaccatta ctgctgccct
     1921 gacaacactt cctccattac caccactgtg cattttggaa aaaggaaggg aggggctctc
     1981 tggggtatgg ggccactgtc ctgtgattct gtctaccaag accctcagtg tttatggctg
     2041 gtcaggacca gggctgggtc tgagttcacc atcagttttg ggctatcgtg gtgcctgagc
     2101 ttgaagctgg gataggaatt aggatcaggt tgtggtttgg actgctcatc agaagagctg
     2161 tctcggggct ggagagatgg ctcagcggtt aagagcgctg actgttcttc tgaaagtcct
     2221 gagttcaaat cccagcaacc acatggtggc tcataaccat ctgtaataag atctgacacc
     2281 ctcttctggt gtctgaagac agctacagtg tacttacata taataataaa taaatctt
//
```

GenBank Submission AF127658

```
Submission 5 of a set of 9 submission(s).

Comment: This cDNA sequence contains Intron alpha.  bp 276 contains the WT
nucleotide.

LOCUS       XXXXX        2547 bp    mRNA            ROD       11-FEB-1999
DEFINITION  Mus musculus (with alternative splicing) RAB geranylgeranyl
            transferase, alpha subunit (Rabggta) mRNA, complete cds
            (alternative splicing adds Intron alpha).
ACCESSION   ;
KEYWORDS    .
SOURCE      house mouse.
  ORGANISM  Mus musculus
            Eukaryota; Metazoa; Chordata; Vertebrata; Mammalia; Eutheria;
            Rodentia; Sciurognathi; Muridae; Murinae; Mus.
REFERENCE   1  (bases 1 to 2547)
  AUTHORS   Detter,J.C., Novack,E.K., Seabra,M, Swank,R.T. and Kingsmore,S.F.
  TITLE     Abnormal Protein Prenylation Leads to Hypopigmentation/Platelet
            Storage Pool Deficiency in the Gunmetal Mouse Mutant
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 2547)
  AUTHORS   Detter,J.C.
  TITLE     Direct Submission
  JOURNAL   Submitted (11-FEB-1999) Molecular Genetics & Microbiology, and
            Medicine, University of Florida, 1600 SW Archer RD, Gainesville,FL
            32610, USA
FEATURES             Location/Qualifiers
     source          1..2547
                     /organism="Mus musculus"
                     /strain="C57BL/6J"
                     /db_xref="taxon:10090"
                     /chromosome="14"
                     /map="between Mit63 and Mit122"
     gene            1..2547
                     /gene="Rabggta"
     CDS             331..2034
                     /gene="Rabggta"
                     /codon_start=1
                     /product="RABGGTA"
                    /translation="MHGRLKVKTSEEQAEAKRLEREQKLKLYQSATQAVFQKREAGEL
                    DESVLELTSQILGANPDFATLWNCRREVLQQLETQKSPEELAALVKAELGFLESCLRV
                    NPKSYGTWHHRCWLLSRLPEPNWARELELCARFLEADERNFHCWDYRRFVAAQAAVAP
                    AEELAFTDSLITRNFSNYSSWHYRSCLLPQLHPQPDSGPQGRLPENVLLRELELVQNA
                    FFTDPNDQSAWFYHRWLLGRAEPHDVLCCLHVSREEACLSVCFSRPLIVGSKMGTLLL
                    TVDEAPLSVEWRTPDGRNRPSHVWLCDLPAASLNDHLPQHTFRVIWTGSDTQKECVLL
                    KGHQECWCRDSATDEQLFRCELSVEKSTVLQSELESCKELQELEPENKWCLLTIILLM
                    RALDPLLYEKETLEYFSTLKAVDPMRAAYLDDLRSKFLVENSVLKMEYADVRVLHLAH
                    KDLTVLCHLEQLLLVTHLDLSHNRLRALPPALAALRCLEVLQASDNVLENLDGVANLP
                    RLRELLLCNNRLQQSAALQTLASCPRLVFLNLQGNSLCQEEGIRERLAEMLPSVSSIL
                    T"
BASE COUNT      521 a    720 c    728 g    578 t
ORIGIN
        1 gtgcaagggt ccacgggacg gacggacggg cgggcgggca ctgctctcta tatgcgcggc
       61 gtgtgcctcg gccctgacgg gtggggcttc ggaaagatcc gtggggacgg aggccgtggt
      121 tgtggtgagc gcttcctcct gacctcacag tgccagccgc cggccccctt ttaactcacg
      181 gggatcctta tcctgggcct gctggcagct tccgggaaac acctgggggg cggggccgtg
      241 tgggcggggt tctgtgattg acatctcttt cggcagattg aacgggctgc ctccggaccc
      301 ctcccctatc gccaacgctg agccgaaacc atgcatgggc gcctgaaggt gaagacgtcc
      361 gaagagcagg cagaggccaa aaggctagag cgggaacaga agctaaagct ctaccagtca
      421 gccactcaag ctgtcttcca gaagcgggag gcaggcgagc tggatgaatc cgtcctagaa
      481 ctgacaagcc agattctggg agccaaccct gattttgcca ccctctggaa ctgtcgcaga
      541 gaagtgctcc agcagctaga aacccagaag tcccctgagg agttggctgc tcttgtgaag
```

```
 601 gcagaactag gcttccttga gagctgtctg cgtgtgaacc ctaagtccta tggcacttgg
 661 caccaccgct gctggctgct gagtcgcctg cctgagccca actgggcccg ggagctggag
 721 ctgtgcgctc gcttcctcga ggccgatgag cggaactttc attgctggga ctatcggcga
 781 tttgtagctg cacaggctgc tgtcgcgcct gcagaggaac tagccttcac tgacagcctc
 841 atcacccgga acttctccaa ctattcttcc tggcattatc gctcctgcct cttgccccag
 901 ctgcaccccc agccagactc tggcccccag ggacggctcc ctgaaaatgt actgctgaga
 961 gagctggagt tggtgcagaa tgccttcttt actgacccca atgatcagag tgcctggttc
1021 tatcaccgct ggctcctggg gcgggcggag ccccacgatg ttctgtgctg cctgcatgtg
1081 agccgggaag aggcctgtct gtcagtctgc ttttctcgtc ccctgatagt gggctccaag
1141 atggggacct tgctactcac ggttgacgag gcacctttga gtgtggaatg gaggactcca
1201 gatggcagga accggcccag ccatgtctgg ctctgtgacc tgcctgctgc ctctctcaat
1261 gaccacttgc cccagcacac gtttcgtgtc atctggacag gaagcgatac ccagaaggag
1321 tgtgtgcttt taaaaggtca ccaggagtgc tggtgccgag actcagccac tgatgaacag
1381 ttgttcaggt gtgagctgtc cgtggagaag tccacagtgc tacagtccga gcttgagtcc
1441 tgtaaggagc tgcaggaact ggagcctgag aataaatggt gcctgctgac catcatcctc
1501 ctgatgaggg cactggaccc cctcctctac gagaaagaaa cactggagta cttcagtacc
1561 ctcaaagctg tggacccgat gagagcagcc tacttggacg acctgcgcag caagttcttg
1621 gtggaaaaca gtgttctcaa gatggagtat gctgacgtgc gtgtgctgca cctggctcac
1681 aaggatctca cagtgctctg ccatttggaa caactgctct tggtcactca tcttgacctg
1741 tcccataatc gtctccgagc cttgccccca gccctggctg ctctgcgctg tctcgaggtg
1801 ttgcaggcca gtgataatgt cctggagaac ttggacggcg tggctaacct tccccggctg
1861 cgggagctgt tactatgtaa taaccgcctc cagcagtctg ctgcacttca gactcttgcc
1921 tcctgcccca ggctagtctt cctcaacctg cagggcaact cgctgtgcca agaagagggc
1981 atccgggagc gtttggctga gatgctaccg tccgtcagca gcatcctcac ctaggaggcc
2041 ccatctcacc ctcacccttt aacttattgg gactgaataa agactggaga ggcccctcta
2101 ggctgccaag ctaccattac tgctgccctg acaacacttc ctccattacc accactgtgc
2161 attttggaaa aaggaaggga ggggctctct ggggtatggg gccactgtcc tgtgattctg
2221 tctaccaaga ccctcagtgt ttatggctgg tcaggaccag ggctgggtct gagttcacca
2281 tcagttttgg gctatcgtgg tgcctgagct tgaagctggg ataggaatta ggatcaggtt
2341 gtggtttgga ctgctcatca gaagagctgt ctcggggctg gagagatggc tcagcggtta
2401 agagcgctga ctgttcttct gaaagtcctg agttcaaatc ccagcaacca catggtggct
2461 cataaccatc tgtaataaga tctgacaccc tcttctggtg tctgaagaca gctacagtgt
2521 acttacatat aataataaat aaatctt
//
```

GenBank Submission AF127659

```
Submission 6 of a set of 9 submission(s).

Comment: This cDNA sequence contains Intron alpha, which contains the pt
mutation, due to alternative splicing.  bp 276 contains the mutant nucleotide.

LOCUS       XXXXX        2547 bp    mRNA            ROD       11-FEB-1999
DEFINITION  Mus musculus (with alternative splicing and pt mutation) RAB
            geranylgeranyl transferase, alpha subunit (Rabggta) mRNA, complete
            cds (alternative splicing adds Intron alpha which contains the pt
            mutation).
ACCESSION   ;
KEYWORDS    .
SOURCE      house mouse.
  ORGANISM  Mus musculus
            Eukaryota; Metazoa; Chordata; Vertebrata; Mammalia; Eutheria;
            Rodentia; Sciurognathi; Muridae; Murinae; Mus.
REFERENCE   1  (bases 1 to 2547)
  AUTHORS   Detter,J.C., Novack,E.K., Seabra,M, Swank,R.T. and Kingsmore,S.F.
  TITLE     Abnormal Protein Prenylation Leads to Hypopigmentation/Platelet
            Storage Pool Deficiency in the Gunmetal Mouse Mutant
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 2547)
  AUTHORS   Detter,J.C.
  TITLE     Direct Submission
  JOURNAL   Submitted (11-FEB-1999) Molecular Genetics & Microbiology, and
            Medicine, University of Florida, 1600 SW Archer RD, Gainesville,FL
            32610, USA
FEATURES             Location/Qualifiers
     source          1..2547
                     /organism="Mus musculus"
                     /strain="C57BL/6J-gm/gm"
                     /db_xref="taxon:10090"
                     /chromosome="14"
                     /map="between Mit63 and Mit122"
     gene            1..2547
                     /gene="Rabggta (with pt mutation)"
     CDS             331..2034
                     /gene="Rabggta (with pt mutation)"
                     /codon_start=1
                     /product="RABGGTA"
                    /translation="MHGRLKVKTSEEQAEAKRLEREQKLKLYQSATQAVFQKREAGEL
                    DESVLELTSQILGANPDFATLWNCRREVLQQLETQKSPEELAALVKAELGFLESCLRV
                    NPKSYGTWHHRCWLLSRLPEPNWARELELCARFLEADERNFHCWDYRRFVAAQAAVAP
                    AEELAFTDSLITRNFSNYSSWHYRSCLLPQLHPQPDSGPQGRLPENVLLRELELVQNA
                    FFTDPNDQSAWFYHRWLLGRAEPHDVLCCLHVSREEACLSVCFSRPLIVGSKMGTLLL
                    TVDEAPLSVEWRTPDGRNRPSHVWLCDLPAASLNDHLPQHTFRVIWTGSDTQKECVLL
                    KGHQECWCRDSATDEQLFRCELSVEKSTVLQSELESCKELQELEPENKWCLLTIILLM
                    RALDPLLYEKETLEYFSTLKAVDPMRAAYLDDLRSKFLVENSVLKMEYADVRVLHLAH
                    KDLTVLCHLEQLLLVTHLDLSHNRLRALPPALAALRCLEVLQASDNVLENLDGVANLP
                    RLRELLLCNNRLQQSAALQTLASCPRLVFLNLQGNSLCQEEGIRERLAEMLPSVSSIL
                    T"
BASE COUNT      522 a    720 c    727 g    578 t
ORIGIN
        1 gtgcaagggt ccacgggacg gacggacggg cgggcgggca ctgctctcta tatgcgcggc
       61 gtgtgcctcg gccctgacgg gtggggcttc ggaaagatcc gtggggacgg aggccgtggt
      121 tgtggtgagc gcttcctcct gacctcacag tgccagccgc cggccccctt ttaactcacg
      181 gggatcctta tcctgggcct gctggcagct tccgggaaac acctgggggg cggggccgtg
      241 tgggcggggt tctgtgattg acatctcttt cggcaaattg aacgggctgc ctccggaccc
      301 ctcccctatc gccaacgctg agccgaaacc atgcatgggc gcctgaaggt gaagacgtcc
      361 gaagagcagg cagaggccaa aaggctagag cgggaacaga agctaaagct ctaccagtca
      421 gccactcaag ctgtcttcca gaagcgggag gcaggcgagc tggatgaatc cgtcctagaa
      481 ctgacaagcc agattctggg agccaaccct gattttgcca ccctctggaa ctgtcgcaga
```

```
     541 gaagtgctcc agcagctaga aacccagaag tcccctgagg agttggctgc tcttgtgaag
     601 gcagaactag gcttccttga gagctgtctg cgtgtgaacc ctaagtccta tggcacttgg
     661 caccaccgct gctggctgct gagtcgcctg cctgagccca actgggcccg ggagctggag
     721 ctgtgcgctc gcttcctcga ggccgatgag cggaactttc attgctggga ctatcggcga
     781 tttgtagctg cacaggctgc tgtcgcgcct gcagaggaac tagccttcac tgacagcctc
     841 atcacccgga acttctccaa ctattcttcc tggcattatc gctcctgcct cttgccccag
     901 ctgcaccccc agccagactc tggcccccag ggacggctcc ctgaaaatgt actgctgaga
     961 gagctggagt tggtgcagaa tgccttcttt actgacccca atgatcagag tgcctggttc
    1021 tatcaccgct ggctcctggg gcgggcggag ccccacgatg ttctgtgctg cctgcatgtg
    1081 agccgggaag aggcctgtct gtcagtctgc ttttctcgtc ccctgatagt gggctccaag
    1141 atggggacct tgctactcac ggttgacgag gcacctttga gtgtggaatg gaggactcca
    1201 gatggcagga accggcccag ccatgtctgg ctctgtgacc tgcctgctgc ctctctcaat
    1261 gaccacttgc cccagcacac gtttcgtgtc atctggacag gaagcgatac ccagaaggag
    1321 tgtgtgcttt taaaaggtca ccaggagtgc tggtgccgag actcagccac tgatgaacag
    1381 ttgttcaggt gtgagctgtc cgtggagaag tccacagtgc tacagtccga gcttgagtcc
    1441 tgtaaggagc tgcaggaact ggagcctgag aataaatggt gcctgctgac catcatcctc
    1501 ctgatgaggg cactggaccc cctcctctac gagaaagaaa cactggagta cttcagtacc
    1561 ctcaaagctg tggacccgat gagagcagcc tacttggacg acctgcgcag caagttcttg
    1621 gtggaaaaca gtgttctcaa gatggagtat gctgacgtgc gtgtgctgca cctggctcac
    1681 aaggatctca cagtgctctg ccatttggaa caactgctct tggtcactca tcttgacctg
    1741 tcccataatc gtctccgagc cttgccccca gccctggctg ctctgcgctg tctcgaggtg
    1801 ttgcaggcca gtgataatgt cctggagaac ttggacggcg tggctaacct tccccggctg
    1861 cgggagctgt tactatgtaa taaccgcctc cagcagtctg ctgcacttca gactcttgcc
    1921 tcctgcccca ggctagtctt cctcaacctg cagggcaact cgctgtgcca agaagagggc
    1981 atccgggagc gtttggctga gatgctaccg tccgtcagca gcatcctcac ctaggaggcc
    2041 ccatctcacc ctcacccttt aacttattgg gactgaataa agactggaga ggcccctcta
    2101 ggctgccaag ctaccattac tgctgccctg acaacacttc ctccattacc accactgtgc
    2161 attttggaaa aaggaaggga ggggctctct ggggtatggg gccactgtcc tgtgattctg
    2221 tctaccaaga ccctcagtgt ttatggctgg tcaggaccag ggctgggtct gagttcacca
    2281 tcagttttgg gctatcgtgg tgcctgagct tgaagctggg ataggaatta ggatcaggtt
    2341 gtggtttgga ctgctcatca gaagagctgt ctcggggctg gagagatggc tcagcggtta
    2401 agagcgctga ctgttcttct gaaagtcctg agttcaaatc ccagcaacca catggtggct
    2461 cataaccatc tgtaataaga tctgacaccc tcttctggtg tctgaagaca gctacagtgt
    2521 acttacatat aataataaat aaatctt
//
```

GenBank Submission AF127660

```
Submission 7 of a set of 9 submission(s).

Comment: This cDNA sequence contains Intron 9 (bp 1186-1282) due to alternative
splicing.  This adds a new STOP.

LOCUS       XXXXX        2492 bp    mRNA            ROD       11-FEB-1999
DEFINITION  Mus musculus (with alternative splicing) RAB geranylgeranyl
            transferase, alpha subunit (Rabggta) mRNA, complete cds
            (alternative splicing adds Intron 9 which contains new STOP
            location).
ACCESSION   ;
KEYWORDS    .
SOURCE      house mouse.
  ORGANISM  Mus musculus
            Eukaryota; Metazoa; Chordata; Vertebrata; Mammalia; Eutheria;
            Rodentia; Sciurognathi; Muridae; Murinae; Mus.
REFERENCE   1  (bases 1 to 2492)
  AUTHORS   Detter,J.C., Novack,E.K., Seabra,M, Swank,R.T. and Kingsmore,S.F.
  TITLE     Abnormal Protein Prenylation Leads to Hypopigmentation/Platelet
            Storage Pool Deficiency in the Gunmetal Mouse Mutant
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 2492)
  AUTHORS   Detter,J.C.
  TITLE     Direct Submission
  JOURNAL   Submitted (11-FEB-1999) Molecular Genetics & Microbiology, and
            Medicine, University of Florida, 1600 SW Archer RD, Gainesville,FL
            32610, USA
FEATURES             Location/Qualifiers
     source          1..2492
                     /organism="Mus musculus"
                     /strain="C57BL/6J"
                     /db_xref="taxon:10090"
                     /chromosome="14"
                     /map="between Mit63 and Mit122"
     gene            1..2492
                     /gene="Rabggta"
     CDS             179..1198
                     /gene="Rabggta"
                     /codon_start=1
                     /product="RABGGTA (alternative)"
                    /translation="MHGRLKVKTSEEQAEAKRLEREQKLKLYQSATQAVFQKREAGEL
                    DESVLELTSQILGANPDFATLWNCRREVLQQLETQKSPEELAALVKAELGFLESCLRV
                    NPKSYGTWHHRCWLLSRLPEPNWARELELCARFLEADERNFHCWDYRRFVAAQAAVAP
                    AEELAFTDSLITRNFSNYSSWHYRSCLLPQLHPQPDSGPQGRLPENVLLRELELVQNA
                    FFTDPNDQSAWFYHRWLLGRAEPHDVLCCLHVSREEACLSVCFSRPLIVGSKMGTLLL
                    TVDEAPLSVEWRTPDGRNRPSHVWLCDLPAASLNDHLPQHTFRVIWTGSDTQKECVLL
                    KGDAV"
BASE COUNT      517 a    716 c    690 g    569 t
ORIGIN
        1 gtgcaagggt ccacgggacg gacggacggg cgggcgggca ctgctctcta tatgcgcggc
       61 gtgtgcctcg gccctgacgg gtggggcttc ggaaagatcc gtggggacgg aggccgtggt
      121 tgtgattgaa cgggctgcct ccggacccct cccctatcgc caacgctgag ccgaaaccat
      181 gcatgggcgc ctgaaggtga agacgtccga agagcaggca gaggccaaaa ggctagagcg
      241 ggaacagaag ctaaagctct accagtcagc cactcaagct gtcttccaga agcgggaggc
      301 aggcgagctg gatgaatccg tcctagaact gacaagccag attctgggag ccaaccctga
      361 ttttgccacc ctctggaact gtcgcagaga agtgctccag cagctagaaa cccagaagtc
      421 ccctgaggag ttggctgctc ttgtgaaggc agaactaggc ttccttgaga gctgtctgcg
      481 tgtgaaccct aagtcctatg gcacttggca ccaccgctgc tggctgctga gtcgcctgcc
      541 tgagcccaac tgggcccggg agctggagct gtgcgctcgc ttcctcgagg ccgatgagcg
      601 gaactttcat tgctgggact atcggcgatt tgtagctgca caggctgctg tcgcgcctgc
      661 agaggaacta gccttcactg acagcctcat cacccggaac ttctccaact attcttcctg
      721 gcattatcgc tcctgcctct tgccccagct gcacccccag ccagactctg gcccccaggg
```

```
 781 acggctccct gaaaatgtac tgctgagaga gctggagttg gtgcagaatg ccttctttac
 841 tgaccccaat gatcagagtg cctggttcta tcaccgctgg ctcctggggc gggcggagcc
 901 ccacgatgtt ctgtgctgcc tgcatgtgag ccgggaagag gcctgtctgt cagtctgctt
 961 ttctcgtccc ctgatagtgg gctccaagat ggggaccttg ctactcacgg ttgacgaggc
1021 acctttgagt gtggaatgga ggactccaga tggcaggaac cggcccagcc atgtctggct
1081 ctgtgacctg cctgctgcct ctctcaatga ccacttgccc cagcacacgt ttcgtgtcat
1141 ctggacagga agcgataccc agaaggagtg tgtgctttta aaaggtgatg ctgtctaaag
1201 ctccccttgc ctcgccagcc ccccagcctt ctctctcctc tccccacagt gtcccatcca
1261 actacttaac ctttccccta ggtcaccagg agtgctggtg ccgagactca gccactgatg
1321 aacagttgtt caggtgtgag ctgtccgtgg agaagtccac agtgctacag tccgagcttg
1381 agtcctgtaa ggagctgcag gaactggagc ctgagaataa atggtgcctg ctgaccatca
1441 tcctcctgat gagggcactg gacccctcc tctacgagaa agaaacactg gagtacttca
1501 gtaccctcaa agctgtggac ccgatgagag cagcctactt ggacgacctg cgcagcaagt
1561 tcttggtgga aaacagtgtt ctcaagatgg agtatgctga cgtgcgtgtg ctgcacctgg
1621 ctcacaagga tctcacagtg ctctgccatt tggaacaact gctcttggtc actcatcttg
1681 acctgtccca taatcgtctc cgagccttgc ccccagccct ggctgctctg cgctgtctcg
1741 aggtgttgca ggccagtgat aatgtcctgg agaacttgga cggcgtggct aaccttcccc
1801 ggctgcggga gctgttacta tgtaataacc gcctccagca gtctgctgca cttcagactc
1861 ttgcctcctg ccccaggcta gtcttcctca acctgcaggg caactcgctg tgccaagaag
1921 agggcatccg ggagcgtttg gctgagatgc taccgtccgt cagcagcatc ctcacctagg
1981 aggccccatc tcaccctcac cctttaactt attgggactg aataaagact ggagaggccc
2041 ctctaggctg ccaagctacc attactgctg ccctgacaac acttcctcca ttaccaccac
2101 tgtgcatttt ggaaaaagga agggaggggc tctctggggt atggggccac tgtcctgtga
2161 ttctgtctac caagaccctc agtgtttatg gctggtcagg accagggctg ggtctgagtt
2221 caccatcagt tttgggctat cgtggtgcct gagcttgaag ctgggatagg aattaggatc
2281 aggttgtggt ttggactgct catcagaaga gctgtctcgg ggctggagag atggctcagc
2341 ggttaagagc gctgactgtt cttctgaaag tcctgagttc aaatcccagc aaccacatgg
2401 tggctcataa ccatctgtaa taagatctga caccctcttc tggtgtctga agacagctac
2461 agtgtactta catataataa taaataaatc tt
//
```

GenBank Submission AF127661

Submission 8 of a set of 9 submission(s).

Comment: This cDNA sequence has lost exon 1, which contains START, due to mutation in intron alpha and also contains Intron 9 (bp 1129-1225) due to alternative splicing. This adds a new STOP.

LOCUS XXXXX 2435 bp mRNA ROD 11-FEB-1999
DEFINITION Mus musculus (with mutation & alternative splicing) RAB geranylgeranyl transferase, alpha subunit (Rabggta) mRNA, complete cds (Mutation causes loss of exon 1 and START) (alternative splicing adds Intron 9 which contains new STOP location).
ACCESSION ;
KEYWORDS .
SOURCE house mouse.
ORGANISM Mus musculus
Eukaryota; Metazoa; Chordata; Vertebrata; Mammalia; Eutheria; Rodentia; Sciurognathi; Muridae; Murinae; Mus.
REFERENCE 1 (bases 1 to 2435)
AUTHORS Detter,J.C., Novack,E.K., Seabra,M, Swank,R.T. and Kingsmore,S.F.
TITLE Abnormal Protein Prenylation Leads to Hypopigmentation/Platelet Storage Pool Deficiency in the Gunmetal Mouse Mutant
JOURNAL Unpublished
REFERENCE 2 (bases 1 to 2435)
AUTHORS Detter,J.C.
TITLE Direct Submission
JOURNAL Submitted (11-FEB-1999) Molecular Genetics & Microbiology, and Medicine, University of Florida, 1600 SW Archer RD, Gainesville,FL 32610, USA
FEATURES Location/Qualifiers
source 1..2435
/organism="Mus musculus"
/strain="C57BL/6J-gm/gm"
/db_xref="taxon:10090"
/chromosome="14"
/map="between Mit63 and Mit122"
BASE COUNT 505 a 693 c 677 g 560 t
ORIGIN

```
        1 gtgcaagggt ccacgggacg gacggacggg cgggcgggca ctgctctcta tatgcgcggc
       61 gtgtgcctcg gccctgacgg gtggggcttc ggaaagatcc gtggggacgg aggccgtggt
      121 tgtgcatggg cgcctgaagg tgaagacgtc cgaagagcag gcagaggcca aaaggctaga
      181 gcgggaacag aagctaaagc tctaccagtc agccactcaa gctgtcttcc agaagcggga
      241 ggcaggcgag ctggatgaat ccgtcctaga actgacaagc cagattctgg gagccaaccc
      301 tgattttgcc accctctgga actgtcgcag agaagtgctc cagcagctag aaacccagaa
      361 gtcccctgag gagttggctg ctcttgtgaa ggcagaacta ggcttccttg agagctgtct
      421 gcgtgtgaac cctaagtcct atggcacttg gcaccaccgc tgctggctgc tgagtcgcct
      481 gcctgagccc aactgggccc gggagctgga gctgtgcgct cgcttcctcg aggccgatga
      541 gcggaacttt cattgctggg actatcggcg atttgtagct gcacaggctg ctgtcgcgcc
      601 tgcagaggaa ctagccttca ctgacagcct catcacccgg aacttctcca actattcttc
      661 ctggcattat cgctcctgcc tcttgcccca gctgcacccc cagccagact ctggccccca
      721 gggacggctc cctgaaaatg tactgctgag agagctggag ttggtgcaga atgccttctt
      781 tactgacccc aatgatcaga gtgcctggtt ctatcaccgc tggctcctgg ggcgggcgga
      841 gccccacgat gttctgtgct gcctgcatgt gagccgggaa gaggcctgtc tgtcagtctg
      901 cttttctcgt cccctgatag tgggctccaa gatggggacc ttgctactca cggttgacga
      961 ggcacctttg agtgtggaat ggaggactcc agatggcagg aaccggccca gccatgtctg
     1021 gctctgtgac ctgcctgctg cctctctcaa tgaccacttg ccccagcaca cgtttcgtgt
     1081 catctggaca ggaagcgata cccagaagga gtgtgtgctt ttaaaaggtg atgctgtcta
     1141 aagctcccct tgcctcgcca gccccccagc cttctctctc ctctccccac agtgtcccat
     1201 ccaactactt aacctttccc ctaggtcacc aggagtgctg gtgccgagac tcagccactg
     1261 atgaacagtt gttcaggtgt gagctgtccg tggagaagtc cacagtgcta cagtccgagc
     1321 ttgagtcctg taaggagctg caggaactgg agcctgagaa taaatggtgc ctgctgacca
     1381 tcatcctcct gatgagggca ctggaccccc tcctctacga gaaagaaaca ctggagtact
     1441 tcagtaccct caaagctgtg gacccgatga gagcagccta cttggacgac ctgcgcagca
```

```
1501 agttcttggt ggaaaacagt gttctcaaga tggagtatgc tgacgtgcgt gtgctgcacc
1561 tggctcacaa ggatctcaca gtgctctgcc atttggaaca actgctcttg gtcactcatc
1621 ttgacctgtc ccataatcgt ctccgagcct tgcccccagc cctggctgct ctgcgctgtc
1681 tcgaggtgtt gcaggccagt gataatgtcc tggagaactt ggacggcgtg gctaaccttc
1741 cccggctgcg ggagctgtta ctatgtaata accgcctcca gcagtctgct gcacttcaga
1801 ctcttgcctc ctgccccagg ctagtcttcc tcaacctgca gggcaactcg ctgtgccaag
1861 aagagggcat ccgggagcgt ttggctgaga tgctaccgtc cgtcagcagc atcctcacct
1921 aggaggcccc atctcaccct cacccttaa cttattggga ctgaataaag actggagagg
1981 cccctctagg ctgccaagct accattactg ctgccctgac aacacttcct ccattaccac
2041 cactgtgcat tttggaaaaa ggaagggagg ggctctctgg ggtatggggc cactgtcctg
2101 tgattctgtc taccaagacc ctcagtgttt atggctggtc aggaccaggg ctgggtctga
2161 gttcaccatc agttttgggc tatcgtggtg cctgagcttg aagctgggat aggaattagg
2221 atcaggttgt ggtttggact gctcatcaga agagctgtct cggggctgga gagatggctc
2281 agcggttaag agcgctgact gttcttctga aagtcctgag ttcaaatccc agcaaccaca
2341 tggtggctca taaccatctg taataagatc tgacaccctc ttctggtgtc tgaagacagc
2401 tacagtgtac ttacatataa taataaataa atctt
//
```

GenBank Submission AF127662

```
Submission 9 of a set of 9 submission(s).

Comment: This cDNA sequence has added the first 84 bp of Intron alpha and lost
the first 13 bp of exon 1 due to cryptic splicing.  It should give the
normal protein b/c the point mutation in Intron alpha has been spliced
out and the start remains.  The new spliced exon 1 goes from bp 124-252.

LOCUS       XXXXX        2466 bp    mRNA            ROD       11-FEB-1999
DEFINITION  Mus musculus (with mutation & cryptic splicing) RAB geranylgeranyl
            transferase, alpha subunit (Rabggta) mRNA, complete cds (cryptic
            splicing adds the first 84bp of Intron alpha and removes the first
            13 bp of exon 1, START remains).
ACCESSION   ;
KEYWORDS    .
SOURCE      house mouse.
  ORGANISM  Mus musculus
            Eukaryota; Metazoa; Chordata; Vertebrata; Mammalia; Eutheria;
            Rodentia; Sciurognathi; Muridae; Murinae; Mus.
REFERENCE   1  (bases 1 to 2466)
  AUTHORS   Detter,J.C., Novack,E.K., Seabra,M, Swank,R.T. and Kingsmore,S.F.
  TITLE     Abnormal Protein Prenylation Leads to Hypopigmentation/Platelet
            Storage Pool Deficiency in the Gunmetal Mouse Mutant
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 2466)
  AUTHORS   Detter,J.C.
  TITLE     Direct Submission
  JOURNAL   Submitted (11-FEB-1999) Molecular Genetics & Microbiology, and
            Medicine, University of Florida, 1600 SW Archer RD, Gainesville,FL
            32610, USA
FEATURES             Location/Qualifiers
     source          1..2466
                     /organism="Mus musculus"
                     /strain="C57BL/6J-gm/gm"
                     /db_xref="taxon:10090"
                     /chromosome="14"
                     /map="between Mit63 and Mit122"
     gene            1..2466
                     /gene="Rabggta (with gunmetal mutation)"
     CDS             250..1953
                     /gene="Rabggta (with gunmetal mutation)"
                     /codon_start=1
                     /product="RABGGTA"
                    /translation="MHGRLKVKTSEEQAEAKRLEREQKLKLYQSATQAVFQKREAGEL
                    DESVLELTSQILGANPDFATLWNCRREVLQQLETQKSPEELAALVKAELGFLESCLRV
                    NPKSYGTWHHRCWLLSRLPEPNWARELELCARFLEADERNFHCWDYRRFVAAQAAVAP
                    AEELAFTDSLITRNFSNYSSWHYRSCLLPQLHPQPDSGPQGRLPENVLLRELELVQNA
                    FFTDPNDQSAWFYHRWLLGRAEPHDVLCCLHVSREEACLSVCFSRPLIVGSKMGTLLL
                    TVDEAPLSVEWRTPDGRNRPSHVWLCDLPAASLNDHLPQHTFRVIWTGSDTQKECVLL
                    KGHQECWCRDSATDEQLFRCELSVEKSTVLQSELESCKELQELEPENKWCLLTIILLM
                    RALDPLLYEKETLEYFSTLKAVDPMRAAYLDDLRSKFLVENSVLKMEYADVRVLHLAH
                    KDLTVLCHLEQLLLVTHLDLSHNRLRALPPALAALRCLEVLQASDNVLENLDGVANLP
                    RLRELLLCNNRLQQSAALQTLASCPRLVFLNLQGNSLCQEEGIRERLAEMLPSVSSIL
                    T"
BASE COUNT      510 a    702 c    695 g    559 t
ORIGIN
        1 gtgcaagggt ccacgggacg gacggacggg cgggcgggca ctgctctcta tatgcgcggc
       61 gtgtgcctcg gccctgacgg gtggggcttc ggaaagatcc gtggggacgg aggccgtggt
      121 tgtggtgagc gcttcctcct gacctcacag tgccagccgc cggccccctt ttaactcacg
      181 gggatcctta tcctgggcct gctggcagcc tccggacccc tcccctatcg ccaacgctga
      241 gccgaaacca tgcatgggcg cctgaaggtg aagacgtccg aagagcaggc agaggccaaa
      301 aggctagagc gggaacagaa gctaaagctc taccagtcag ccactcaagc tgtcttccag
      361 aagcgggagg caggcgagct ggatgaatcc gtcctagaac tgacaagcca gattctggga
```

```
 421 gccaaccctg attttgccac cctctggaac tgtcgcagag aagtgctcca gcagctagaa
 481 acccagaagt cccctgagga gttggctgct cttgtgaagg cagaactagg cttccttgag
 541 agctgtctgc gtgtgaaccc taagtcctat ggcacttggc accaccgctg ctggctgctg
 601 agtcgcctgc ctgagcccaa ctgggcccgg gagctggagc tgtgcgctcg cttcctcgag
 661 gccgatgagc ggaactttca ttgctgggac tatcggcgat ttgtagctgc acaggctgct
 721 gtcgcgcctg cagaggaact agccttcact gacagcctca tcacccggaa cttctccaac
 781 tattcttcct ggcattatcg ctcctgcctc ttgccccagc tgcaccccca gccagactct
 841 ggcccccagg gacggctccc tgaaaatgta ctgctgagag agctggagtt ggtgcagaat
 901 gccttcttta ctgaccccaa tgatcagagt gcctggttct atcaccgctg gctcctgggg
 961 cgggcggagc cccacgatgt tctgtgctgc ctgcatgtga gccgggaaga ggcctgtctg
1021 tcagtctgct tttctcgtcc cctgatagtg ggctccaaga tggggacctt gctactcacg
1081 gttgacgagg cacctttgag tgtggaatgg aggactccag atggcaggaa ccggcccagc
1141 catgtctggc tctgtgacct gcctgctgcc tctctcaatg accacttgcc ccagcacacg
1201 tttcgtgtca tctggacagg aagcgatacc cagaaggagt gtgtgctttt aaaaggtcac
1261 caggagtgct ggtgccgaga ctcagccact gatgaacagt tgttcaggtg tgagctgtcc
1321 gtggagaagt ccacagtgct acagtccgag cttgagtcct gtaaggagct gcaggaactg
1381 gagcctgaga ataaatggtg cctgctgacc atcatcctcc tgatgagggc actggacccc
1441 ctcctctacg agaaagaaac actggagtac ttcagtaccc tcaaagctgt ggacccgatg
1501 agagcagcct acttggacga cctgcgcagc aagttcttgg tggaaaacag tgttctcaag
1561 atggagtatg ctgacgtgcg tgtgctgcac ctggctcaca aggatctcac agtgctctgc
1621 catttggaac aactgctctt ggtcactcat cttgacctgt cccataatcg tctccgagcc
1681 ttgcccccag ccctggctgc tctgcgctgt ctcgaggtgt tgcaggccag tgataatgtc
1741 ctggagaact tggacggcgt ggctaacctt ccccggctgc gggagctgtt actatgtaat
1801 aaccgcctcc agcagtctgc tgcacttcag actcttgcct cctgccccag gctagtcttc
1861 ctcaacctgc agggcaactc gctgtgccaa gaagagggca tccgggagcg tttggctgag
1921 atgctaccgt ccgtcagcag catcctcacc taggaggccc catctcaccc tcacccttta
1981 acttattggg actgaataaa gactggagag gcccctctag gctgccaagc taccattact
2041 gctgccctga caacacttcc tccattacca ccactgtgca ttttggaaaa aggaagggag
2101 gggctctctg gggtatgggg ccactgtcct gtgattctgt ctaccaagac cctcagtgtt
2161 tatggctggt caggaccagg gctgggtctg agttcaccat cagttttggg ctatcgtggt
2221 gcctgagctt gaagctggga taggaattag gatcaggttg tggtttggac tgctcatcag
2281 aagagctgtc tcggggctgg agagatggct cagcggttaa gagcgctgac tgttcttctg
2341 aaagtcctga gttcaaatcc cagcaaccac atggtggctc ataaccatct gtaataagat
2401 ctgacaccct cttctggtgt ctgaagacag ctacagtgta cttacatata ataataaata
2461 aatctt
//
```

Rabggta Sequence Comparisons of *gm/gm* and +/+ (DNA and cDNA)

Comparisons of *Rabggta* DNA and mRNA from *gm/gm* and B6 (control) mice. This Appendix complements the RT-PCR fragments shown in Figure 4.4. The two sets of 20 black italic nucleotides shown in this sequence represents the locations of the primers used to amplify the fragments seen in Figure 4.4. All sequence originated from the *Rabggta* gene. **Sequence 1** corresponds to B6 DNA (contains all exons but only intron alpha for space saving purposes). **Sequence 2** corresponds to *gm/gm* DNA (contains all exons but only intron alpha for space saving purposes). **Sequence 3** corresponds to B6 mRNA (see Figure 4.4b, band A). **Sequence 4** corresponds to *gm/gm* mRNA (see Figure 4.4b, band C). **Sequence 5** corresponds to *gm/gm* mRNA that has undergone cryptic splicing (see Figure 4.4b, band D). The three spaces (...) before a bolded letter identifies the exon/intron boundaries. The underlined "ATG" represents the start codon. The underlined "TAG" represents the stop codon. Genomic sequences 1 & 2 only differ (green "g" and red "a") at the point mutation shown in Figure 4,4c. The mutation occurs at the last nucleotide of intron alpha's splice acceptor. Coding sequences 3 & 4 differ at exon 1. B6 DNA (# 1) undergoes normal splicing, and thus retains exon 1 (green sequence in # 3) along with its "ATG". *gm/gm* DNA (# 2) does not undergo normal splicing due to the pt mutation, and thus skips/splices out exon 1 (red asterisks in # 4) causing the loss of its "ATG". Coding sequence 5 has utilized cryptic splice sites 70 bp from the 3' end of intron alpha and 13 bp from the 5' end of exon 1. This region is identified by the blue sequence. The "ATG" is retained in this *gm/gm* fragment.

1. ***GTGCAAGGGTCCACGGGAC***GGACGGACGGGCGGGCGGGCACTGCTCTCTATATGCGCGGCGTGTGCCTC
2. ***GTGCAAGGGTCCACGGGAC***GGACGGACGGGCGGGCGGGCACTGCTCTCTATATGCGCGGCGTGTGCCTC

3. ***GTGCAAGGGTCCACGGGAC***GGACGGACGGGCGGGCGGGCACTGCTCTCTATATGCGCGGCGTGTGCCTC
4. ***GTGCAAGGGTCCACGGGAC***GGACGGACGGGCGGGCGGGCACTGCTCTCTATATGCGCGGCGTGTGCCTC
5. ***GTGCAAGGGTCCACGGGAC***GGACGGACGGGCGGGCGGGCACTGCTCTCTATATGCGCGGCGTGTGCCTC

1. GGCCCTGACGGGTGGGGCTTCGGAAAGATCCGTGGGGACGGAGGCCGTGGTTGTGgtgagcgcttcctcctgacctcac
2. GGCCCTGACGGGTGGGGCTTCGGAAAGATCCGTGGGGACGGAGGCCGTGGTTGTGgtgagcgcttcctcctgacctcac

3. GGCCCTGACGGGTGGGGCTTCGGAAAGATCCGTGGGGACGGAGGCCGTGGTTGTG.....
4. GGCCCTGACGGGTGGGGCTTCGGAAAGATCCGTGGGGACGGAGGCCGTGGTTGTG.....
5. GGCCCTGACGGGTGGGGCTTCGGAAAGATCCGTGGGGACGGAGGCCGTGGTTGTGgtgagcgcttcctcctgacctcac

1. agtgccagccgccggcccccttttaactcacggggatccttatcctgggcctgctggcagcttccgggaaacacctgggggcggggccgtgtgggcggggttctgtgatt
2. agtgccagccgccggcccccttttaactcacggggatccttatcctgggcctgctggcagcttccgggaaacacctgggggcggggccgtgtgggcggggttctgtgatt

3. .....
4. .....
5. agtgccagccgccggcccccttttaactcacggggatccttatcctgggcctgctggcag.....

1. gacatctctttcggcagATTGAACGGGCTGCCTCCGGACCCCTCCCCTATCGCCAACGCTGAGCCGAAACCATG...C
2. gacatctctttcggcaaATTGAACGGGCTGCCTCCGGACCCCTCCCCTATCGCCAACGCTGAGCCGAAACCATG...C

3. ....ATTGAACGGGCTGCCTCCGGACCCCTCCCCTATCGCCAACGCTGAGCCGAAACCATG...C
4. .... **********...C
5. ....CCTCCGGACCCCTCCCCTATCGCCAACGCTGAGCCGAAACCATG...C

1. ATGGGCGCCTGAAGGTGAAGACGTCCGAAGAGCAGGCAGAGGCCAAAAGGCTAGAGCGGGAACAGAAG
2. ATGGGCGCCTGAAGGTGAAGACGTCCGAAGAGCAGGCAGAGGCCAAAAGGCTAGAGCGGGAACAGAAG

3. ATGGGCGCCTGAAGGTGAAGACGTCCGAAGAGCAGGCAGAGGCCAAAAGGCTAGAGCGGGAACAGAAG
4. ATGGGCGCCTGAAGGTGAAGACGTCCGAAGAGCAGGCAGAGGCCAAAAGGCTAGAGCGGGAACAGAAG
5. ATGGGCGCCTGAAGGTGAAGACGTCCGAAGAGCAGGCAGAGGCCAAAAGGCTAGAGCGGGAACAGAAG

1. CTAAAGCTCTACCAGTCAGCCACTCAAGCTGTCTTCCAGAAG...CGGGAGGCAGGCGAGCTGGATGAATC
2. CTAAAGCTCTACCAGTCAGCCACTCAAGCTGTCTTCCAGAAG...CGGGAGGCAGGCGAGCTGGATGAATC

3. CTAAAGCTCTACCAGTCAGCCACTCAAGCTGTCTTCCAGAAG...CGGGAGGCAGGCGAGCTGGATGAATC
4. CTAAAGCTCTACCAGTCAGCCACTCAAGCTGTCTTCCAGAAG...CGGGAGGCAGGCGAGCTGGATGAATC
5. CTAAAGCTCTACCAGTCAGCCACTCAAGCTGTCTTCCAGAAG...CGGGAGGCAGGCGAGCTGGATGAATC

1. CGTCCTAGAACTGACAAGCCAGATTCTGGGAGCCAACCCTGATTTTGCCACCCTCTGGAACTGTCGCAGA
2. CGTCCTAGAACTGACAAGCCAGATTCTGGGAGCCAACCCTGATTTTGCCACCCTCTGGAACTGTCGCAGA

3. CGTCCTAGAACTGACAAGCCAGATTCTGGGAGCCAACCCTGATTTTGCCACCCTCTGGAACTGTCGCAGA
4. CGTCCTAGAACTGACAAGCCAGATTCTGGGAGCCAACCCTGATTTTGCCACCCTCTGGAACTGTCGCAGA
5. CGTCCTAGAACTGACAAGCCAGATTCTGGGAGCCAACCCTGATTTTGCCACCCTCTGGAACTGTCGCAGA

1. GAAGTGCTCCAGCAGCTAGAAACCCAGAAGT...CCCCTGAGGAGTTGGCTGCTCTTGTGAAGGCAGAACT
2. GAAGTGCTCCAGCAGCTAGAAACCCAGAAGT...CCCCTGAGGAGTTGGCTGCTCTTGTGAAGGCAGAACT

3. GAAGTGCTCCAGCAGCTAGAAACCCAGAAGT...CCCCTGAGGAGTTGGCTGCTCTTGTGAAGGCAGAACT
4. GAAGTGCTCCAGCAGCTAGAAACCCAGAAGT...CCCCTGAGGAGTTGGCTGCTCTTGTGAAGGCAGAACT
5. GAAGTGCTCCAGCAGCTAGAAACCCAGAAGT...CCCCTGAGGAGTTGGCTGCTCTTGTGAAGGCAGAACT

1. AGGCTTCCTT***GAGAGCTGTCTGCGTGTGAA***CCCTAAGTCCTATGGCACTTGGCACCACCGCTGCTGGCTGC
2. AGGCTTCCTT***GAGAGCTGTCTGCGTGTGAA***CCCTAAGTCCTATGGCACTTGGCACCACCGCTGCTGGCTGC

3. AGGCTTCCTT***GAGAGCTGTCTGCGTGTGAA***CCCTAAGTCCTATGGCACTTGGCACCACCGCTGCTGGCTGC
4. AGGCTTCCTT***GAGAGCTGTCTGCGTGTGAA***CCCTAAGTCCTATGGCACTTGGCACCACCGCTGCTGGCTGC
5. AGGCTTCCTT***GAGAGCTGTCTGCGTGTGAA***CCCTAAGTCCTATGGCACTTGGCACCACCGCTGCTGGCTGC

1. TGAGTCGCCTGCCTGAGCCCAACTGGGCCCGGGAGCTGGAGCTGTGCGCTCGCTTCCTCGAGGCCGATGA
2. TGAGTCGCCTGCCTGAGCCCAACTGGGCCCGGGAGCTGGAGCTGTGCGCTCGCTTCCTCGAGGCCGATGA

3. TGAGTCGCCTGCCTGAGCCCAACTGGGCCCGGGAGCTGGAGCTGTGCGCTCGCTTCCTCGAGGCCGATGA
4. TGAGTCGCCTGCCTGAGCCCAACTGGGCCCGGGAGCTGGAGCTGTGCGCTCGCTTCCTCGAGGCCGATGA
5. TGAGTCGCCTGCCTGAGCCCAACTGGGCCCGGGAGCTGGAGCTGTGCGCTCGCTTCCTCGAGGCCGATGA

1. GCGGAACT...TTCATTGCTGGGACTATCGGCGATTTGTAGCTGCACAGGCTGCTGTCGCGCCTGCAGAGG
2. GCGGAACT...TTCATTGCTGGGACTATCGGCGATTTGTAGCTGCACAGGCTGCTGTCGCGCCTGCAGAGG

3. GCGGAACT...TTCATTGCTGGGACTATCGGCGATTTGTAGCTGCACAGGCTGCTGTCGCGCCTGCAGAGG
4. GCGGAACT...TTCATTGCTGGGACTATCGGCGATTTGTAGCTGCACAGGCTGCTGTCGCGCCTGCAGAGG
5. GCGGAACT...TTCATTGCTGGGACTATCGGCGATTTGTAGCTGCACAGGCTGCTGTCGCGCCTGCAGAGG

1. AACTAGCCTTCACTGACAGCCTCATCACCCGGAACTTCTCCAACTATTCTTCCTGGCATTATCGCTCCTGC
2. AACTAGCCTTCACTGACAGCCTCATCACCCGGAACTTCTCCAACTATTCTTCCTGGCATTATCGCTCCTGC

3. AACTAGCCTTCACTGACAGCCTCATCACCCGGAACTTCTCCAACTATTCTTCCTGGCATTATCGCTCCTGC
4. AACTAGCCTTCACTGACAGCCTCATCACCCGGAACTTCTCCAACTATTCTTCCTGGCATTATCGCTCCTGC
5. AACTAGCCTTCACTGACAGCCTCATCACCCGGAACTTCTCCAACTATTCTTCCTGGCATTATCGCTCCTGC

1. CTCTTGCCCCAGCTGCACCCCCAGCCAGACTCTGGCCCCCAGGGACGGCTCCCTGAAAATGTACTGCTGA
2. CTCTTGCCCCAGCTGCACCCCCAGCCAGACTCTGGCCCCCAGGGACGGCTCCCTGAAAATGTACTGCTGA

3. CTCTTGCCCCAGCTGCACCCCCAGCCAGACTCTGGCCCCCAGGGACGGCTCCCTGAAAATGTACTGCTGA
4. CTCTTGCCCCAGCTGCACCCCCAGCCAGACTCTGGCCCCCAGGGACGGCTCCCTGAAAATGTACTGCTGA
5. CTCTTGCCCCAGCTGCACCCCCAGCCAGACTCTGGCCCCCAGGGACGGCTCCCTGAAAATGTACTGCTGA

1. GAG...AGCTGGAGTTGGTGCAGAATGCCTTCTTTACTGACCCCAATGATCAGAGTGCCTGGTTCTATCACC
2. GAG...AGCTGGAGTTGGTGCAGAATGCCTTCTTTACTGACCCCAATGATCAGAGTGCCTGGTTCTATCACC

3. GAG...AGCTGGAGTTGGTGCAGAATGCCTTCTTTACTGACCCCAATGATCAGAGTGCCTGGTTCTATCACC
4. GAG...AGCTGGAGTTGGTGCAGAATGCCTTCTTTACTGACCCCAATGATCAGAGTGCCTGGTTCTATCACC
5. GAG...AGCTGGAGTTGGTGCAGAATGCCTTCTTTACTGACCCCAATGATCAGAGTGCCTGGTTCTATCACC

1. GCTGGCTCCTGGGGCG...GGCGGAGCCCCACGATGTTCTGTGCTGCCTGCATGTGAGCCGGGAAGAGGCC
2. GCTGGCTCCTGGGGCG...GGCGGAGCCCCACGATGTTCTGTGCTGCCTGCATGTGAGCCGGGAAGAGGCC
3. GCTGGCTCCTGGGGCG...GGCGGAGCCCCACGATGTTCTGTGCTGCCTGCATGTGAGCCGGGAAGAGGCC
4. GCTGGCTCCTGGGGCG...GGCGGAGCCCCACGATGTTCTGTGCTGCCTGCATGTGAGCCGGGAAGAGGCC
5. GCTGGCTCCTGGGGCG...GGCGGAGCCCCACGATGTTCTGTGCTGCCTGCATGTGAGCCGGGAAGAGGCC

1. TGTCTGTCAGTCTGCTTTTCTCGTCCCCTGATAGTG...GGCTCCAAGATGGGGACCTTGCTACTCACGGTTG
2. TGTCTGTCAGTCTGCTTTTCTCGTCCCCTGATAGTG...GGCTCCAAGATGGGGACCTTGCTACTCACGGTTG
3. TGTCTGTCAGTCTGCTTTTCTCGTCCCCTGATAGTG...GGCTCCAAGATGGGGACCTTGCTACTCACGGTTG
4. TGTCTGTCAGTCTGCTTTTCTCGTCCCCTGATAGTG...GGCTCCAAGATGGGGACCTTGCTACTCACGGTTG
5. TGTCTGTCAGTCTGCTTTTCTCGTCCCCTGATAGTG...GGCTCCAAGATGGGGACCTTGCTACTCACGGTTG

1. ACGAGGCACCTTTGAGTGTGGAATGGAGGACTCCAGATGGCAGGAACCGGCCCAGCCATGTCTGG...CTC
2. ACGAGGCACCTTTGAGTGTGGAATGGAGGACTCCAGATGGCAGGAACCGGCCCAGCCATGTCTGG...CTC
3. ACGAGGCACCTTTGAGTGTGGAATGGAGGACTCCAGATGGCAGGAACCGGCCCAGCCATGTCTGG...CTC
4. ACGAGGCACCTTTGAGTGTGGAATGGAGGACTCCAGATGGCAGGAACCGGCCCAGCCATGTCTGG...CTC
5. ACGAGGCACCTTTGAGTGTGGAATGGAGGACTCCAGATGGCAGGAACCGGCCCAGCCATGTCTGG...CTC

1. TGTGACCTGCCTGCTGCCTCTCTCAATGACCACTTGCCCCAGCACACGTTTCGTGTCATCTGGACAGGAAG
2. TGTGACCTGCCTGCTGCCTCTCTCAATGACCACTTGCCCCAGCACACGTTTCGTGTCATCTGGACAGGAAG
3. TGTGACCTGCCTGCTGCCTCTCTCAATGACCACTTGCCCCAGCACACGTTTCGTGTCATCTGGACAGGAAG
4. TGTGACCTGCCTGCTGCCTCTCTCAATGACCACTTGCCCCAGCACACGTTTCGTGTCATCTGGACAGGAAG
5. TGTGACCTGCCTGCTGCCTCTCTCAATGACCACTTGCCCCAGCACACGTTTCGTGTCATCTGGACAGGAAG

1. CGATACCCAGAAGGAGTGTGTGCTTTTAAAAGG...TCACCAGGAGTGCTGGTGCCGAGACTCAGCCACTG
2. CGATACCCAGAAGGAGTGTGTGCTTTTAAAAGG...TCACCAGGAGTGCTGGTGCCGAGACTCAGCCACTG
3. CGATACCCAGAAGGAGTGTGTGCTTTTAAAAGG...TCACCAGGAGTGCTGGTGCCGAGACTCAGCCACTG
4. CGATACCCAGAAGGAGTGTGTGCTTTTAAAAGG...TCACCAGGAGTGCTGGTGCCGAGACTCAGCCACTG
5. CGATACCCAGAAGGAGTGTGTGCTTTTAAAAGG...TCACCAGGAGTGCTGGTGCCGAGACTCAGCCACTG

1. ATGAACAGTTGTTCAGGTG...TGAGCTGTCCGTGGAGAAGTCCACAGTGCTACAGTCCGAGCTTGAGTCC
2. ATGAACAGTTGTTCAGGTG...TGAGCTGTCCGTGGAGAAGTCCACAGTGCTACAGTCCGAGCTTGAGTCC
3. ATGAACAGTTGTTCAGGTG...TGAGCTGTCCGTGGAGAAGTCCACAGTGCTACAGTCCGAGCTTGAGTCC
4. ATGAACAGTTGTTCAGGTG...TGAGCTGTCCGTGGAGAAGTCCACAGTGCTACAGTCCGAGCTTGAGTCC
5. ATGAACAGTTGTTCAGGTG...TGAGCTGTCCGTGGAGAAGTCCACAGTGCTACAGTCCGAGCTTGAGTCC

1. TGTAAGGAGCTGCAGGAACTGGAGCCTGAGAATAAATG...GTGCCTGCTGACCATCATCCTCCTGATGAG
2. TGTAAGGAGCTGCAGGAACTGGAGCCTGAGAATAAATG...GTGCCTGCTGACCATCATCCTCCTGATGAG
3. TGTAAGGAGCTGCAGGAACTGGAGCCTGAGAATAAATG...GTGCCTGCTGACCATCATCCTCCTGATGAG
4. TGTAAGGAGCTGCAGGAACTGGAGCCTGAGAATAAATG...GTGCCTGCTGACCATCATCCTCCTGATGAG
5. TGTAAGGAGCTGCAGGAACTGGAGCCTGAGAATAAATG...GTGCCTGCTGACCATCATCCTCCTGATGAG

1. GGCACTGGACCCCCTCCTCTACGAGAAAGAAACACTGGAGTACTTCAGTACCCTCAAAGC...TGTGGACC
2. GGCACTGGACCCCCTCCTCTACGAGAAAGAAACACTGGAGTACTTCAGTACCCTCAAAGC...TGTGGACC
3. GGCACTGGACCCCCTCCTCTACGAGAAAGAAACACTGGAGTACTTCAGTACCCTCAAAGC...TGTGGACC
4. GGCACTGGACCCCCTCCTCTACGAGAAAGAAACACTGGAGTACTTCAGTACCCTCAAAGC...TGTGGACC
5. GGCACTGGACCCCCTCCTCTACGAGAAAGAAACACTGGAGTACTTCAGTACCCTCAAAGC...TGTGGACC

1. CGATGAGAGCAGCCTACTTGGACGACCTGCGCAGCAAGTTCTTGGTGGAAAACAGTGTTCTCAAGATGG
2. CGATGAGAGCAGCCTACTTGGACGACCTGCGCAGCAAGTTCTTGGTGGAAAACAGTGTTCTCAAGATGG
3. CGATGAGAGCAGCCTACTTGGACGACCTGCGCAGCAAGTTCTTGGTGGAAAACAGTGTTCTCAAGATGG
4. CGATGAGAGCAGCCTACTTGGACGACCTGCGCAGCAAGTTCTTGGTGGAAAACAGTGTTCTCAAGATGG
5. CGATGAGAGCAGCCTACTTGGACGACCTGCGCAGCAAGTTCTTGGTGGAAAACAGTGTTCTCAAGATGG

1. AGTATGCTGACGTGCGTGTGCTGCACCTGGCTCACAAGG...ATCTCACAGTGCTCTGCCATTTGGAACAAC
2. AGTATGCTGACGTGCGTGTGCTGCACCTGGCTCACAAGG...ATCTCACAGTGCTCTGCCATTTGGAACAAC
3. AGTATGCTGACGTGCGTGTGCTGCACCTGGCTCACAAGG...ATCTCACAGTGCTCTGCCATTTGGAACAAC
4. AGTATGCTGACGTGCGTGTGCTGCACCTGGCTCACAAGG...ATCTCACAGTGCTCTGCCATTTGGAACAAC
5. AGTATGCTGACGTGCGTGTGCTGCACCTGGCTCACAAGG...ATCTCACAGTGCTCTGCCATTTGGAACAAC

1. TGCTCTTGGTCACTCATCTTGACCTGTCCCATAATCGTCTCCGAGCCTTGCCCCCAGCCCTGGCTGCTCTG
2. TGCTCTTGGTCACTCATCTTGACCTGTCCCATAATCGTCTCCGAGCCTTGCCCCCAGCCCTGGCTGCTCTG

3. TGCTCTTGGTCACTCATCTTGACCTGTCCCATAATCGTCTCCGAGCCTTGCCCCCAGCCCTGGCTGCTCTG
4. TGCTCTTGGTCACTCATCTTGACCTGTCCCATAATCGTCTCCGAGCCTTGCCCCCAGCCCTGGCTGCTCTG
5. TGCTCTTGGTCACTCATCTTGACCTGTCCCATAATCGTCTCCGAGCCTTGCCCCCAGCCCTGGCTGCTCTG

1. CGCTGTCTCGAGGT...**G**TTGCAGGCCAGTGATAATGTCCTGGAGAACTTGGACGGCGTGGCTAACCTTCCC
2. CGCTGTCTCGAGGT...**G**TTGCAGGCCAGTGATAATGTCCTGGAGAACTTGGACGGCGTGGCTAACCTTCCC

3. CGCTGTCTCGAGGT...**G**TTGCAGGCCAGTGATAATGTCCTGGAGAACTTGGACGGCGTGGCTAACCTTCCC
4. CGCTGTCTCGAGGT...**G**TTGCAGGCCAGTGATAATGTCCTGGAGAACTTGGACGGCGTGGCTAACCTTCCC
5. CGCTGTCTCGAGGT...**G**TTGCAGGCCAGTGATAATGTCCTGGAGAACTTGGACGGCGTGGCTAACCTTCCC

1. CGGCTGCGGGAGCTGTTACTATGTAATAACC...**G**CCTCCAGCAGTCTGCTGCACTTCAGACTCTTGCCTCC
2. CGGCTGCGGGAGCTGTTACTATGTAATAACC...**G**CCTCCAGCAGTCTGCTGCACTTCAGACTCTTGCCTCC

3. CGGCTGCGGGAGCTGTTACTATGTAATAACC...**G**CCTCCAGCAGTCTGCTGCACTTCAGACTCTTGCCTCC
4. CGGCTGCGGGAGCTGTTACTATGTAATAACC...**G**CCTCCAGCAGTCTGCTGCACTTCAGACTCTTGCCTCC
5. CGGCTGCGGGAGCTGTTACTATGTAATAACC...**G**CCTCCAGCAGTCTGCTGCACTTCAGACTCTTGCCTCC

1. TGCCCCAGGCTAGTCTTCCTCAACCTGCAGGGCAACTCGCTGTGCCAAGAAGAGGGCATCCGGGAGCGTT
2. TGCCCCAGGCTAGTCTTCCTCAACCTGCAGGGCAACTCGCTGTGCCAAGAAGAGGGCATCCGGGAGCGTT

3. TGCCCCAGGCTAGTCTTCCTCAACCTGCAGGGCAACTCGCTGTGCCAAGAAGAGGGCATCCGGGAGCGTT
4. TGCCCCAGGCTAGTCTTCCTCAACCTGCAGGGCAACTCGCTGTGCCAAGAAGAGGGCATCCGGGAGCGTT
5. TGCCCCAGGCTAGTCTTCCTCAACCTGCAGGGCAACTCGCTGTGCCAAGAAGAGGGCATCCGGGAGCGTT

1. TGGCTGAGATGCTACCGTCCGTCAGCAGCATCCTCACC**TAG**GAGGCCCCATCTCACCCTCACCCTTTAAC
2. TGGCTGAGATGCTACCGTCCGTCAGCAGCATCCTCACC**TAG**GAGGCCCCATCTCACCCTCACCCTTTAAC

3. TGGCTGAGATGCTACCGTCCGTCAGCAGCATCCTCACC**TAG**GAGGCCCCATCTCACCCTCACCCTTTAAC
4. TGGCTGAGATGCTACCGTCCGTCAGCAGCATCCTCACC**TAG**GAGGCCCCATCTCACCCTCACCCTTTAAC
5. TGGCTGAGATGCTACCGTCCGTCAGCAGCATCCTCACC**TAG**GAGGCCCCATCTCACCCTCACCCTTTAAC

1. TTATTGGGACTGAATAAAGACTGGAGAGGCCCCTCTAGGCTGCCAAGCTACCATTACTGCTGCCCTGACA
2. TTATTGGGACTGAATAAAGACTGGAGAGGCCCCTCTAGGCTGCCAAGCTACCATTACTGCTGCCCTGACA

3. TTATTGGGACTGAATAAAGACTGGAGAGGCCCCTCTAGGCTGCCAAGCTACCATTACTGCTGCCCTGACA
4. TTATTGGGACTGAATAAAGACTGGAGAGGCCCCTCTAGGCTGCCAAGCTACCATTACTGCTGCCCTGACA
5. TTATTGGGACTGAATAAAGACTGGAGAGGCCCCTCTAGGCTGCCAAGCTACCATTACTGCTGCCCTGACA

1. ACACTTCCTCCATTACCACCACTGTGCATTTTGGAAAAAGGAAGGGAGGGGCTCTCTGGGGTATGGGGCC
2. ACACTTCCTCCATTACCACCACTGTGCATTTTGGAAAAAGGAAGGGAGGGGCTCTCTGGGGTATGGGGCC

3. ACACTTCCTCCATTACCACCACTGTGCATTTTGGAAAAAGGAAGGGAGGGGCTCTCTGGGGTATGGGGCC
4. ACACTTCCTCCATTACCACCACTGTGCATTTTGGAAAAAGGAAGGGAGGGGCTCTCTGGGGTATGGGGCC
5. ACACTTCCTCCATTACCACCACTGTGCATTTTGGAAAAAGGAAGGGAGGGGCTCTCTGGGGTATGGGGCC

1. ACTGTCCTGTGATTCTGTCTACCAAGACCCTCAGTGTTTATGGCTGGTCAGGACCAGGGCTGGGTCTGAGT
2. ACTGTCCTGTGATTCTGTCTACCAAGACCCTCAGTGTTTATGGCTGGTCAGGACCAGGGCTGGGTCTGAGT

3. ACTGTCCTGTGATTCTGTCTACCAAGACCCTCAGTGTTTATGGCTGGTCAGGACCAGGGCTGGGTCTGAGT
4. ACTGTCCTGTGATTCTGTCTACCAAGACCCTCAGTGTTTATGGCTGGTCAGGACCAGGGCTGGGTCTGAGT
5. ACTGTCCTGTGATTCTGTCTACCAAGACCCTCAGTGTTTATGGCTGGTCAGGACCAGGGCTGGGTCTGAGT

1. TCACCATCAGTTTTGGGCTATCGTGGTGCCTGAGCTTGAAGCTGGGATAGGAATTAGGATCAGGTTGTGG
2. TCACCATCAGTTTTGGGCTATCGTGGTGCCTGAGCTTGAAGCTGGGATAGGAATTAGGATCAGGTTGTGG

3. TCACCATCAGTTTTGGGCTATCGTGGTGCCTGAGCTTGAAGCTGGGATAGGAATTAGGATCAGGTTGTGG
4. TCACCATCAGTTTTGGGCTATCGTGGTGCCTGAGCTTGAAGCTGGGATAGGAATTAGGATCAGGTTGTGG
5. TCACCATCAGTTTTGGGCTATCGTGGTGCCTGAGCTTGAAGCTGGGATAGGAATTAGGATCAGGTTGTGG

1. TTTGGACTGCTCATCAGAAGAGCTGTCTCGGGGCTGGAGAGATGGCTCAGCGGTTAAGAGCGCTGACTGT
2. TTTGGACTGCTCATCAGAAGAGCTGTCTCGGGGCTGGAGAGATGGCTCAGCGGTTAAGAGCGCTGACTGT

3. TTTGGACTGCTCATCAGAAGAGCTGTCTCGGGGCTGGAGAGATGGCTCAGCGGTTAAGAGCGCTGACTGT
4. TTTGGACTGCTCATCAGAAGAGCTGTCTCGGGGCTGGAGAGATGGCTCAGCGGTTAAGAGCGCTGACTGT
5. TTTGGACTGCTCATCAGAAGAGCTGTCTCGGGGCTGGAGAGATGGCTCAGCGGTTAAGAGCGCTGACTGT

1. TCTTCTGAAAGTCCTGAGTTCAAATCCCAGCAACCACATGGTGGCTCATAACCATCTGTAATAAGATCTG
2. TCTTCTGAAAGTCCTGAGTTCAAATCCCAGCAACCACATGGTGGCTCATAACCATCTGTAATAAGATCTG
3. TCTTCTGAAAGTCCTGAGTTCAAATCCCAGCAACCACATGGTGGCTCATAACCATCTGTAATAAGATCTG
4. TCTTCTGAAAGTCCTGAGTTCAAATCCCAGCAACCACATGGTGGCTCATAACCATCTGTAATAAGATCTG
5. TCTTCTGAAAGTCCTGAGTTCAAATCCCAGCAACCACATGGTGGCTCATAACCATCTGTAATAAGATCTG

1. ACACCCTCTTCTGGTGTCTGAAGACAGCTACAGTGTACTTACATATAATAATAAATAAATCTT
2. ACACCCTCTTCTGGTGTCTGAAGACAGCTACAGTGTACTTACATATAATAATAAATAAATCTT
3. ACACCCTCTTCTGGTGTCTGAAGACAGCTACAGTGTACTTACATATAATAATAAATAAATCTT
4. ACACCCTCTTCTGGTGTCTGAAGACAGCTACAGTGTACTTACATATAATAATAAATAAATCTT
5. ACACCCTCTTCTGGTGTCTGAAGACAGCTACAGTGTACTTACATATAATAATAAATAAATCTT

APPENDIX B
INTER-SPECIES *RABGGTA* SEQUENCE AND AMINO ACID COMPARISONS

Inter-species *Rabggta* coding sequence comparisons. **a,** Mouse (Query) vs. Rat (Subject) sequence alignment. Over the 1704 coding nucleotides, mouse and rat match 94%. **b,** Mouse (Query) vs. Human (Subject) sequence alignment. Over the 1704 coding nucleotides, mouse and human match 86%.

(a) Mouse vs. Rat Sequence

```
gb|L10415|RATRABGERA Rat rab geranylgeranyl transferase alpha-subunit mRNA,
complete cds.
            Length = 2660

 Score = 2601 bits (1312), Expect = 0.0
 Identities = 1606/1704 (94%)
 Strand = Plus / Plus


Query: 1    atgcatgggcgcctgaaggtgaagacgtccgaagagcaggcagaggccaaaaggctagag 60
            ||||||||||||||||||||||||||||| ||||||||||||||||||||||||||||||
Sbjct: 51   atgcatgggcgcctgaaggtgaagacgtcggaagagcaggcagaggccaaaaggctagaa 110


Query: 61   cgggaacagaagctaaagctctaccagtcagccactcaagctgtcttccagaagcgggag 120
            || || ||||||||||||||||||||||||||||||||||||||||||||||||||  ||
Sbjct: 111  cgagagcagaagctaaagctctaccagtcagccactcaagctgtcttccagaagcgtcag 170


Query: 121  gcaggcgagctggatgaatccgtcctagaactgacaagccagattctgggagccaaccct 180
            || || ||||||||||||||| || ||||||||||||||||||||||||||||||||||||
Sbjct: 171  gctggtgagctggatgaatcagtgctagaactgacaagccagattctgggagccaaccct 230


Query: 181  gattttgccaccctctggaactgtcgcagagaagtgctccagcagctagaaacccagaag 240
            |||||||||||||||||||||||||| |||||||| |||||||| || |||||| |||||
Sbjct: 231  gattttgccaccctctggaactgtcgaagagaagtactccagcacctggaaaccgagaag 290


Query: 241  tcccctgaggagttggctgctcttgtgaaggcagaactaggcttccttgagagctgtctg 300
            ||||||||||||  ||||||||||||||||||||||| |||||||| ||||||||||||
Sbjct: 291  tcccctgaggagtcagctgctcttgtgaaggcagaactgggcttcctagagagctgtctt 350


Query: 301  cgtgtgaaccctaagtcctatggcacttggcaccaccgctgctggctgctgagtcgcctg 360
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 351  cgtgtgaaccctaagtcctatggcacttggcaccaccgctgctggctgctgagtcgcctg 410
```

```
Query: 361  cctgagcccaactgggcccgggagctggagctgtgcgctcgcttcctcgaggccgatgag 420
            ||||||||||||||||||||||||||||||||| ||||||||||||||||||||||||||
Sbjct: 411  cctgagcccaactgggcccgggagctggagctatgcgctcgcttcctcgaggccgatgag 470

Query: 421  cggaactttcattgctgggactatcggcgatttgtagctgcacaggctgctgtcgcgcct 480
            |||||||||||||||||||||||||||||||||||||| ||||||||||||| || |||
Sbjct: 471  cggaactttcattgctgggactatcggcgatttgtagccgcacaggctgctgttgcccct 530

Query: 481  gcagaggaactagccttcactgacagcctcatcacccggaacttctccaactattcttcc 540
            |||||||||||||||||||||||||||||||||||||| |||||||||||||||| |||
Sbjct: 531  gcagaggaactagccttcactgacagcctcatcacccgtaacttctccaactattcctcc 590

Query: 541  tggcattatcgctcctgcctcttgccccagctgcacccccagccagactctggcccccag 600
            |||||||||||||||||||||||| ||||||||||||||||||||||||||||||| |||
Sbjct: 591  tggcattatcgctcctgcctcttgccacagctgcacccccagccagactctggcccacag 650

Query: 601  ggacggctccctgaaaatgtactgctgagagagctggagttggtgcagaatgccttcttt 660
            || |||||||||||||||||||||||| ||||||||||||||||||||||||||||||
Sbjct: 651  ggtcggctccctgaaaatgtactgctgaaagagctggagttggtgcagaatgccttcttt 710

Query: 661  actgaccccaatgatcagagtgcctggttctatcaccgctggctcctggggcgggcggag 720
            |||||||||||| ||||||||||||||||||||||||||||||||||||| |||||||||
Sbjct: 711  actgaccccaacgatcagagtgcctggttctatcaccgctggctcctgggtcgggcggag 770

Query: 721  ccccacgatgttctgtgctgcctgcatgtgagccgggaagaggcctgtctgtcagtctgc 780
            |||||||||||||| |||||  |||||||||||||||| |||||||||||||| ||||||
Sbjct: 771  ccccacgatgttctatgctgtgtgcatgtgagccgggaggaggcctgtctgtcggtctgc 830

Query: 781  ttttctcgtcccctgatagtgggctccaagatggggaccttgctactcacggttgacgag 840
            |||||||||||||||| ||||||||||  |||||||||||||||||| |||||| |||
Sbjct: 831  ttttctcgtcccctgacagtgggctccagaatggggaccttgctactcatggttgatgag 890

Query: 841  gcacctttgagtgtggaatggaggactccagatggcaggaaccggcccagccatgtctgg 900
            |||||||||||||||| ||||||||||||||||||||||||||||||||||||||||||
Sbjct: 891  gcacctttgagtgtggagtggaggactccagatggcaggaaccggcccagccatgtctgg 950

Query: 901  ctctgtgacctgcctgctgcctctctcaatgaccacttgccccagcacacgtttcgtgtc 960
            |||||||||||||||||||||||||||||||| ||||||||||||||||||||||||||
Sbjct: 951  ctctgtgacctgcctgctgcctctctcaatgaccagttgccccagcacacgtttcgtgtc 1010

Query: 961  atctggacaggaagcgatacccagaaggagtgtgtgcttttaaaaggtcaccaggagtgc 1020
            |||||||||||||| |||| |||||||||||||||||||||||  | ||  || |||
Sbjct: 1011 atctggacaggaagtgatagccagaaggagtgtgtgcttttaaaagaccgcccagaatgc 1070

Query: 1021 tggtgccgagactcagccactgatgaacagttgttcaggtgtgagctgtccgtggagaag 1080
            |||||||||||||||||||||||||||||| | |||||||||||||||| ||||| |||
Sbjct: 1071 tggtgccgagactcagccactgatgaacagctattcaggtgtgagctgtcagtggaaaag 1130

Query: 1081 tccacagtgctacagtccgagcttgagtcctgtaaggagctgcaggaactggagcctgag 1140
            |||||||||||| ||||| |||||||| ||||||||||||||||||||| |||||||||
Sbjct: 1131 tccacagtgctgcagtctgagcttgaatcctgtaaggagctgcaggaactagagcctgag 1190
```

```
Query: 1141 aataaatggtgcctgctgaccatcatcctcctgatgagggcactggacccccctcctctac 1200
            |||||||||||||||||||||||||||||| |||||| | ||||||||||||||||||||
Sbjct: 1191 aataaatggtgcctgctgaccatcatcctgctgatgcgagcactggacccccctcctctat 1250


Query: 1201 gagaaagaaacactggagtacttcagtaccctcaaagctgtggacccgatgagagcagcc 1260
            ||||| ||||||||| |||||||||||||||||||||||||||||| ||||||||||
Sbjct: 1251 gagaaggaaacactgcagtacttcagtaccctcaaagctgtggacccaatgagagcagct 1310


Query: 1261 tacttggacgacctgcgcagcaagttcttggtggaaaacagtgttctcaagatggagtat 1320
            |||||||| |||||||||||||||||||| |||| ||||||||||||||||||||||
Sbjct: 1311 tacttggatgacctgcgcagcaagttcttgctggagaacagtgttctcaagatggagtac 1370


Query: 1321 gctgacgtgcgtgtgctgcacctggctcacaaggatctcacagtgctctgccatttggaa 1380
            ||||| || || ||||||||||||||||||||||||||||||||||||||||  |||||
Sbjct: 1371 gctgatgtacgcgtgctgcacctggctcacaaggatctcacagtgctctgccacctggaa 1430


Query: 1381 caactgctcttggtcactcatcttgacctgtcccataatcgtctccgagccttgccccca 1440
            |||||||||||||||||||||||||||||||||||||||| |||||||||| ||||||
Sbjct: 1431 caactgctcttggtcactcatcttgacctgtcccataatcgactccgagccttaccccca 1490


Query: 1441 gccctggctgctctgcgctgtctcgaggtgttgcaggccagtgataatgtcctggagaac 1500
            |||||||||||||||||||||| |||||| |||||||||||| |||| ||||||||||
Sbjct: 1491 gccctggctgctctgcgctgtcttgaggtgctgcaggccagtgacaatgccctggagaac 1550


Query: 1501 ttggacggcgtggctaaccttccccggctgcgggagctgttactatgtaataaccgcctc 1560
             |||| || |||||||||||||||||||| |  |||||||||||||||||||||||||||
Sbjct: 1551 gtggatggtgtggctaaccttccccggctacaagagctgttactatgtaataaccgcctc 1610


Query: 1561 cagcagtctgctgcacttcagactcttgcctcctgccccaggctagtcttcctcaacctg 1620
            || |||||||| ||| | ||| |||||| |||||||||||||||||| ||||||||||
Sbjct: 1611 caacagtctgcagcaatccagcctcttgtctcctgccccaggctagtcctcctcaacctg 1670


Query: 1621 cagggcaactcgctgtgccaagaagagggcatccgggagcgtttggctgagatgctaccg 1680
            |||||||||||||||||||||||||||||||||| |||||||||||||||||||||||
Sbjct: 1671 cagggcaactcgctgtgccaagaagagggcatccaggagcgtttggctgagatgctaccg 1730


Query: 1681 tccgtcagcagcatcctcacctag 1704
            || ||||||||||||||||||||
Sbjct: 1731 tctgtcagcagcatcctcacctag 1754
```

(b) Mouse vs. Human Sequence

```
ref|NM_004581.1|RABGGTA| Homo sapiens Rab geranylgeranyltransferase, alpha
subunit (RABGGTA)
            mRNA >gi|2950169|emb|Y08200|HSRABGTRA Homo sapiens mRNA
            for rab geranylgeranyl transferase, alpha-subunit
            Length = 2067

 Score = 1505 bits (759), Expect = 0.0
 Identities = 1467/1703 (86%)
 Strand = Plus / Plus


Query: 1    atgcatgggcgcctgaaggtgaagacgtccgaagagcaggcagaggccaaaaggctagag 60
            ||||| || ||||||||||||||||||| |||||||||| ||||||||||||||||||
Sbjct: 275  atgcacggacgcctgaaggtgaagacgtcagaagagcaggcggaggccaaaaggctagag 334


Query: 61   cgggaacagaagctaaagctctaccagtcagccactcaagctgtcttccagaagcgggag 120
            || || |||||||| ||||| ||||||||||||| || || || ||||||||||  ||
Sbjct: 335  cgagagcagaagctgaagctataccagtcagccacccaggccgtattccagaagcgccag 394


Query: 121  gcaggcgagctggatgaatccgtcctagaactgacaagccagattctgggagccaaccct 180
            || || |||||||||||| ||||| || ||||||||||||||||||||||||||||||||
Sbjct: 395  gctggtgagctggatgagtccgtgctggaactgacaagccagattctgggagccaaccct 454


Query: 181  gattttgccaccctctggaactgtcgcagagaagtgctccagcagctagaaacccagaag 240
            |||||||||||||||||||||| ||  |||| |||||||||||||| || || ||||||
Sbjct: 455  gattttgccaccctctggaactgccgacgagaggtgctccagcagctggagactcagaag 514


Query: 241  tcccctgaggagttggctgctcttgtgaaggcagaactaggcttccttgagagctgtctg 300
            || ||||| |||||||||||||| |||||||||||||| |||||||| |||||||| |||
Sbjct: 515  tctcctgaagagttggctgctctggtgaaggcagaactgggcttcctggagagctgcctg 574


Query: 301  cgtgtgaaccctaagtcctatggcacttggcaccaccgctgctggctgctgagtcgcctg 360
            || |||||||| ||||| ||||| || |||||||||| |||||||||| | ||||||
Sbjct: 575  cgggtgaaccccaagtcttatggtacctggcaccaccgatgctggctgctaggccgcctg 634


Query: 361  cctgagcccaactgggcccgggagctggagctgtgcgctcgcttcctcgaggccgatgag 420
            |||||||||||||| |||| |||||||||| || || || ||||| ||||  ||||||
Sbjct: 635  cctgagcccaactggacccgagagctggagctctgtgcccgtttcctggaggtggatgag 694


Query: 421  cggaactttcattgctgggactatcggcgatttgtagctgcacaggctgctgtcgcgcct 480
            |||||||||| |||||||||||||||| ||||| ||  ||||||| || ||  | |||
Sbjct: 695  cggaactttcactgctgggactatcggcggtttgtggccacacaggcagccgtgccccct 754


Query: 481  gcagaggaactagccttcactgacagcctcatcacccggaacttctccaactattcttcc 540
            ||||| || |||||||||||||||||||||||||| |||||||||||||| ||||||
Sbjct: 755  gcagaagagctagccttcactgacagcctcatcacccgaaacttctccaactactcttcc 814


Query: 541  tggcattatcgctcctgcctcttgccccagctgcacccccagccagactctggcccccag 600
            |||||||| |||||||| |||||||||||| |||||||||||||| || ||||| || |||
Sbjct: 815  tggcattaccgctcctgtctcttgccccagttgcacccccagccggattctggaccacag 874
```

```
Query: 601  ggacggctccctgaaaatgtactgctgagagagctggagttggtgcagaatgccttctttt 660
             || || ||||||||  |||| ||||| | |||||||||| ||||||||||||||||||
Sbjct: 875  gggcgcctccctgaggatgtgctgctcaaagagctggagctggtgcagaatgccttcttc 934

Query: 661  actgaccccaatgatcagagtgcctggttctatcaccgctggctcctggggcgggcggag 720
             |||||||||||||| |||||||||||||| |||||||| |||||||| || || || ||
Sbjct: 935  actgaccccaatgaccagagtgcctggttttatcaccggtggctcctaggtcgagctgac 994

Query: 721  ccccacgatgttctgtgctgcctgcatgtgagccgggaagaggcctgtctgtcagtctgc 780
             ||||| ||||  ||| |||| |||||||||||||||||| |||||||||||  | |||| |
Sbjct: 995  ccccaggatgcactgcgctgtctgcatgtgagccgggacgaggcctgtctgactgtctcc 1054

Query: 781  ttttctcgtcccctgatagtgggctccaagatggggaccttgctactcacggttgacgag 840
             || ||||| |||||  |||||||||||| ||||| || |||||| |||| |||||| ||
Sbjct: 1055 ttctctcggccctcttagtgggctccaggatggagatcttgctgctcatggttgatgat 1114

Query: 841  gcacctttgagtgtggaatggaggactccagatggcaggaaccggcccagccatgtctgg 900
              | ||  ||| |||||| |||||||| ||||||||||||||||||||||||||||||||
Sbjct: 1115 tctcccctgattgtggagtggaggaccccagatggcaggaaccggcccagccatgtctgg 1174

Query: 901  ctctgtgacctgcctgctgcctctctcaatgaccacttgccccagcacacgtttcgtgtc 960
             |||||||||||||||||||||| ||||| ||||| |||||||| || || ||||| |||
Sbjct: 1175 ctctgtgacctgcctgctgcctccctcaacgaccagttgccccaacatacatttcgcgtc 1234

Query: 961  atctggacaggaagcgatacccagaaggagtgtgtgcttttaaaaggtcaccaggagtgc 1020
             || ||||||| | |||||  |||||| || || |||||||||||||| | ||||||| ||
Sbjct: 1235 atttggacagcaggcgatgtccagaaagaatgcgtgcttttaaaaggccgccaggagggc 1294

Query: 1021 tggtgccgagactcagccactgatgaacagttgttcaggtgtgagctgtccgtggagaag 1080
             |||||||| |||||  | || || || ||| | |||||||||||||||| |||||||||
Sbjct: 1295 tggtgccgggactccacgacagacgagcagctattcaggtgtgagctgtcagtggagaag 1354

Query: 1081 tccacagtgctacagtccgagcttgagtcctgtaaggagctgcaggaactggagcctgag 1140
             |||||||||||| ||||| ||||| || |||||||||||||||||| ||||||||||||
Sbjct: 1355 tccacagtgctgcagtctgagctggaatcctgtaaggagctgcaggagctggagcctgag 1414

Query: 1141 aataaatggtgcctgctgaccatcatcctcctgatgagggcactggacccccctcctctac 1200
             |||||||||||||||| |||||||||| |||||| |||||||||||||||| || ||
Sbjct: 1415 aataaatggtgcctgcttaccatcatcctgctgatgcgggcactggacccccctgctgtat 1474

Query: 1201 gagaaagaaacactggagtacttcagtaccctcaaagctgtggacccgatgagagcagcc 1260
             ||||| || || ||| |||||||   |||||||| || |||||||| ||| | ||| |
Sbjct: 1475 gagaaggagaccctgcagtacttccagaccctcaaggccgtggaccccatgcgggcaacg 1534

Query: 1261 tacttggacgacctgcgcagcaagttcttggtggaaaacagtgttctcaagatggagtat 1320
             ||  |||| |||||||||||||||||| |||| || || || ||||||||||||||||
Sbjct: 1535 tatctggatgacctgcgcagcaagttcttgctggagaatagcgtgctcaagatggagtat 1594

Query: 1321 gctgacgtgcgtgtgctgcacctggctcacaaggatctcacagtgctctgccatttggaa 1380
             || || |||||||||||||||||||||||||||||| |||||||||||||| ||||||
Sbjct: 1595 gccgaggtgcgtgtgctgcacctggctcacaaggatctgacagtgctctgccatctggaa 1654
```

```
Query: 1381 caactgctcttggtcactcatcttgacctgtcccataatcgtctccgagccttgccccca 1440
            || ||||||||||||||| ||||||||| |||| || ||||| |||||| || |||| ||
Sbjct: 1655 cagctgctcttggtcacccatcttgacttgtcacacaatcgcctccgaaccctgccacct 1714


Query: 1441 gccctggctgctctgcgctgtctcgaggtgttgcaggccagtgataatgtcctggagaac 1500
            || |||||||| |||||||| || |||||| |||||||||||||||||    | |||  |
Sbjct: 1715 gcactggctgccctgcgctgccttgaggtgctgcaggccagtgataatgcaatagagtcc 1774


Query: 1501 ttggacggcgtggctaaccttccccggctgcgggagctgttactatgtaataaccgcctc 1560
             ||||||||||  | ||||| |||||||||| ||||||| |||| || || |||||||||
Sbjct: 1775 ctggacggcgtcaccaacctaccccggctgcaggagctgctactgtgcaacaaccgcctc 1834


Query: 1561 cagcagtctgctgcacttcagactcttgcctcctgccccaggctagtcttcctcaacctg 1620
            |||||| |||| |  || ||| |||||||||||||||||||| ||| ||||||||||
Sbjct: 1835 cagcagcctgcagtgctccagcctcttgcctcctgccccaggctggtcctcctcaacctg 1894


Query: 1621 cagggcaactcgctgtgccaagaagagggcatccgggagcgtttggctgagatgctaccg 1680
            ||||| ||| |||||||||||  | |||||||  |||||   |||||||  |||| ||
Sbjct: 1895 cagggtaacccgctgtgccaagcggtgggcatcttggagcaactggctgaactgctgcct 1954


Query: 1681 tccgtcagcagcatcctcaccta 1703
            || || |||||| ||||||||||
Sbjct: 1955 tcagttagcagcgtcctcaccta 1977
```

Inter-species Rabggta protein comparisons. **a,** Mouse (top line) vs. Rat (bottom line) protein alignment. Over the 567 amino acids, mouse and rat match 95.8%. No large regional differences are observed. **b,** Mouse (top line) vs. Human (bottom line) protein alignment. Over the 567 amino acids, mouse and human match 90.8%. No large regional differences are observed. **c,** Mouse (top line) vs. Yeast BET4 (bottom line) protein alignment. Over 290 amino acids, mouse and yeast match 33%.

(a) Mouse vs. Rat Amino Acid

```
sp|Q08602|PGTA_RAT RAB GERANYLGERANYLTRANSFERASE ALPHA SUBUNIT (RAB
          GERANYL-GERANYLTRANSFERASE ALPHA SUBUNIT) (RAB GG
          TRANSFERASE) (RAB GGTASE)
          Length = 567

 Score = 1033 bits (2643), Expect = 0.0
 Identities = 543/567 (96%), Positives = 554/567 (98%)

Query: 1   MHGRLKVKTSEEQAEAKRLEREQKLKLYQSATQAVFQKREAGELDESVLELTSQILGANP 60
           MHGRLKVKTSEEQAEAKRLEREQKLKLYQSATQAVFQKR+AGELDESVLELTSQILGANP
Sbjct: 1   MHGRLKVKTSEEQAEAKRLEREQKLKLYQSATQAVFQKRQAGELDESVLELTSQILGANP 60

Query: 61  DFATLWNCRREVLQQLETQKSPEELAALVKAELGFLESCLRVNPKSYGTWHHRCWLLSRL 120
           DFATLWNCRREVLQ LET+KSPEE AALVKAELGFLESCLRVNPKSYGTWHHRCWLLSRL
Sbjct: 61  DFATLWNCRREVLQHLETEKSPEESAALVKAELGFLESCLRVNPKSYGTWHHRCWLLSRL 120

Query: 121 PEPNWARELELCARFLEADERNFHCWDYRRFVAAQAAVAPAEELAFTDSLITRNFSNYSS 180
           PEPNWARELELCARFLEADERNFHCWDYRRFVAAQAAVAPAEELAFTDSLITRNFSNYSS
Sbjct: 121 PEPNWARELELCARFLEADERNFHCWDYRRFVAAQAAVAPAEELAFTDSLITRNFSNYSS 180

Query: 181 WHYRSCLLPQLHPQPDSGPQGRLPENVLLRELELVQNAFFTDPNDQSAWFYHRWLLGRAE 240
           WHYRSCLLPQLHPQPDSGPQGRLPENVLL+ELELVQNAFFTDPNDQSAWFYHRWLLGRAE
Sbjct: 181 WHYRSCLLPQLHPQPDSGPQGRLPENVLLKELELVQNAFFTDPNDQSAWFYHRWLLGRAE 240

Query: 241 PHDVLCCLHVSREEACLSVCFSRPLIVGSKMGTLLLTVDEAPLSVEWRTPDGRNRPSHVW 300
           PHDVLCC+HVSREEACLSVCFSRPL VGS+MGTLLL VDEAPLSVEWRTPDGRNRPSHVW
Sbjct: 241 PHDVLCCVHVSREEACLSVCFSRPLTVGSRMGTLLLMVDEAPLSVEWRTPDGRNRPSHVW 300

Query: 301 LCDLPAASLNDHLPQHTFRVIWTGSDTQKECVLLKGHQECWCRDSATDEQLFRCELSVEK 360
           LCDLPAASLND LPQHTFRVIWTGSD+QKECVLLK   ECWCRDSATDEQLFRCELSVEK
Sbjct: 301 LCDLPAASLNDQLPQHTFRVIWTGSDSQKECVLLKDRPECWCRDSATDEQLFRCELSVEK 360

Query: 361 STVLQSELESCKELQELEPENKWCLLTIILLMRALDPLLYEKETLEYFSTLKAVDPMRAA 420
           STVLQSELESCKELQELEPENKWCLLTIILLMRALDPLLYEKETL+YFSTLKAVDPMRAA
Sbjct: 361 STVLQSELESCKELQELEPENKWCLLTIILLMRALDPLLYEKETLQYFSTLKAVDPMRAA 420

Query: 421 YLDDLRSKFLVENSVLKMEYADVRVLHLAHKDLTVLCHLEQLLLVTHLDLSHNRLRALPP 480
           YLDDLRSKFL+ENSVLKMEYADVRVLHLAHKDLTVLCHLEQLLLVTHLDLSHNRLRALPP
Sbjct: 421 YLDDLRSKFLLENSVLKMEYADVRVLHLAHKDLTVLCHLEQLLLVTHLDLSHNRLRALPP 480

Query: 481 ALAALRCLEVLQASDNVLENLDGVANLPRLRELLLCNNRLQQSAALQTLASCPRLVFLNL 540
           ALAALRCLEVLQASDN LEN+DGVANLPRL+ELLLCNNRLQQSAA+Q L SCPRLV LNL
Sbjct: 481 ALAALRCLEVLQASDNALENVDGVANLPRLQELLLCNNRLQQSAAIQPLVSCPRLVLLNL 540

Query: 541 QGNSLCQEEGIRERLAEMLPSVSSILT 567
           QGNSLCQEEGI+ERLAEMLPSVSSILT
Sbjct: 541 QGNSLCQEEGIQERLAEMLPSVSSILT 567
```

(b) Mouse vs. Human Amino Acid

```
sp|Q92696|PGTA_HUMAN RAB GERANYLGERANYLTRANSFERASE ALPHA SUBUNIT (RAB
          GERANYL-GERANYLTRANSFERASE ALPHA SUBUNIT) (RAB GG
          TRANSFERASE) (RAB GGTASE)
          Length = 567

 Score =  977 bits (2499), Expect = 0.0
 Identities = 514/567 (91%), Positives = 535/567 (94%)

Query: 1   MHGRLKVKTSEEQAEAKRLEREQKLKLYQSATQAVFQKREAGELDESVLELTSQILGANP 60
           MHGRLKVKTSEEQAEAKRLEREQKLKLYQSATQAVFQKR+AGELDESVLELTSQILGANP
Sbjct: 1   MHGRLKVKTSEEQAEAKRLEREQKLKLYQSATQAVFQKRQAGELDESVLELTSQILGANP 60

Query: 61  DFATLWNCRREVLQQLETQKSPEELAALVKAELGFLESCLRVNPKSYGTWHHRCWLLSRL 120
           DFATLWNCRREVLQQLETQKSPEELAALVKAELGFLESCLRVNPKSYGTWHHRCWLL RL
Sbjct: 61  DFATLWNCRREVLQQLETQKSPEELAALVKAELGFLESCLRVNPKSYGTWHHRCWLLGRL 120

Query: 121 PEPNWARELELCARFLEADERNFHCWDYRRFVAAQAAVAPAEELAFTDSLITRNFSNYSS 180
           PEPNW RELELCARFLE DERNFHCWDYRRFVA+QAAV PAEELAFTDSLITRNFSNYSS
Sbjct: 121 PEPNWTRELELCARFLEVDERNFHCWDYRRFVATQAAVPPAEELAFTDSLITRNFSNYSS 180

Query: 181 WHYRSCLLPQLHPQPDSGPQGRLPENVLLRELELVQNAFFTDPNDQSAWFYHRWLLGRAE 240
           WHYRSCLLPQLHPQPDSGPQGRLPE+VLL+ELELVQNAFFTDPNDQSAWFYHRWLLGRA+
Sbjct: 181 WHYRSCLLPQLHPQPDSGPQGRLPEDVLLKELELVQNAFFTDPNDQSAWFYHRWLLGRAD 240

Query: 241 PHDVLCCLHVSREEACLSVCFSRPLIVGSKMGTLLLTVDEAPLSVEWRTPDGRNRPSHVW 300
           P D L CLHVSR+EACL+V FSRPL+VGS+M  LLL VD++PL VEWRTPDGRNRPSHVW
Sbjct: 241 PQDALRCLHVSRDEACLTVSFSRPLLVGSRMEILLLMVDDSPLIVEWRTPDGRNRPSHVW 300

Query: 301 LCDLPAASLNDHLPQHTFRVIWTGSDTQKECVLLKGHQECWCRDSATDEQLFRCELSVEK 360
           LCDLPAASLND LPQHTFRVIWT  D QKECVLLKG QE WCRDS TDEQLFRCELSVEK
Sbjct: 301 LCDLPAASLNDQLPQHTFRVIWTAGDVQKECVLLKGRQEGWCRDSTTDEQLFRCELSVEK 360

Query: 361 STVLQSELESCKELQELEPENKWCLLTIILLMRALDPLLYEKETLEYFSTLKAVDPMRAA 420
           STVLQSELESCKELQELEPENKWCLLTIILLMRALDPLLYEKE L+YF TLKAVDPMRA
Sbjct: 361 STVLQSELESCKELQELEPENKWCLLTIILLMRALDPLLYEKERLQYFQTLKAVDPMRAT 420

Query: 421 YLDDLRSKFLVENSVLKMEYADVRVLHLAHKDLTVLCHLEQLLLVTHLDLSHNRLRALPP 480
           YLDDLRSKFL+ENSVLKMEYA+VRVLHLAHKDLTVLCHLEQLLLVTHLDLSHNRLR+LPP
Sbjct: 421 YLDDLRSKFLLENSVLKMEYAEVRVLHLAHKDLTVLCHLEQLLLVTHLDLSHNRLRTLPP 480

Query: 481 ALAALRCLEVLQASDNVLENLDGVANLPRLRELLLCNNRLQQSAALQTLASCPRLVFLNL 540
           ALAALRCLEVLQASDN +E+L+GV+NLPRL+ELLLCNNRLQQ A LQ LASCPRLV LNL
Sbjct: 481 ALAALRCLEVLQASDNAIESLEGVTNLPRLQELLLCNNRLQQPAVLQPLASCPRLVLLNL 540

Query: 541 QGNSLCQEEGIRERLAEMLPSVSSILT 567
           QGN LCQ  GI E+LAE+LPSVSS+LT
Sbjct: 541 QGNPLCQAVGILEQLAELLPSVSSVLT 567
```

(c) Mouse vs. Yeast Amino Acid

```
sp|Q00618|BET4_YEAST TYPE II PROTEINS GERANYLGERANYLTRANSFERASE ALPHA SUBUNIT
(TYPE II
           PROTEIN GERANYL-GERANYLTRANSFERASE ALPHA SUBUNIT)
           (GGTASE-II-ALPHA) (PGGT) (YPT1/SEC4 PROTEINS
           GERANYLGERANYLTRANSFERASE ALPHA SUBUNIT)
           Length = 290

 Score =  117 bits (291), Expect = 4e-26
 Identities = 68/206 (33%), Positives = 103/206 (50%), Gaps = 20/206 (9%)

Query: 49  LELTSQILGANPDFATLWNCRREVLQQLETQKSPEELAALVKAELGFLESCLRVNPKSYG 108
           L+ TS++L  NP+F  +WN RR+++  L ++            EL F+   L+  PK Y
Sbjct: 12  LKKTSELLEKNPEFNAIWNYRRDIIASLASELE----IPFWDKELVFVMMLLKDYPKVYW 67

Query: 109 TWHHRCWLLSRLPEPN---WARELELCARFLEADERNFHCWDYRRFVAAQAAVAPA---- 165
            W+HR W+L   P  +   W  EL +  + LE D RN+H W YRR V  +
Sbjct: 68  IWNHRLWVLKHYPTSSPKVWQTELAVVNKLLEQDARNYHGWHYRRIVVGNIESITNKSLD 127

Query: 166 -EELAFTDSLITRNFSNYSSWHYRSCLLPQLHPQPDSGPQGRLPENVLLRELELVQNAFF 220
            EE+ +T   I  N SNYS+WH R  ++ ++  + + G Q    +  +  E+  + NA F
Sbjct: 128 KEEFEYTTIKINNNISNYSAWHQRVQIISRMFQKGEVGNQ----KEYIRTEISYIINAMF 183

Query: 221 TDPNDQSAWFYHRWLLGRAEPHDVLC 246
           TD  DQS WFY +W +     +D++C
Sbjct: 184 TDAEDQSVWFYIKWFI----KNDIVC 205
```

REFERENCES

1. Zauli G, Catani L. (1995) Human megakaryocyte biology and pathophysiology. *Crit. Rev. Oncol. Hematol.* 21:135-157.

2. Bennett JS, Kolodziej MA. (1992) Disorders of platelet function. *Dis. Mon.* 38:577-631.

3. Brass LF, Manning DR, Shattil SJ. (1990) GTP-binding proteins and platelet activation. *Prog. Hemost. Thromb.* 127-175.

4. Corash L, Chen HY, Levin J, Baker G, Lu H, Mok Y. (1987) Regulation of thrombopoiesis; effects of degree of thrombocytopenia on megakaryocyte ploidy and platelet volume. *Blood* 85:402-413.

5. Dexter TM, Spooncer E. (1987) Growth and differentiation in the hemopoietic system. *Annu. Rev. Cell Biol.* 3:423-441.

6. Thompson CB, Eaton KA, Princiotta SM, Rushin CA, Valeri CR. (1982) Size dependent platelet subpopulations: relationship of platelet volume to ultrastructure, enzymatic activity, and function. *Br. J. Haematol.* 50:509-519.

7. Bennett JS. (1992) Mechanisms of platelet adhesion and aggregation. An update. *Hosp. Pract.* 27:124.

8. Bauer C, Wuillemin W. (1996) Blood, Plasma proteins, coagulation, fibrinolysis, and thrombocyte Function. "Comprehensive Human Physiology," Springer, Berlin. vol. 2, pp1651-1675.

9. Hayward CPM, Kelton JG. (1995) Multimerein: a multimeric protein stored in platelet α-granules. *Platelets* 6:1-10.

10. Fuse I. (1996) Disorders of platelet function. *Crit. Rev. Oncol. Hematol.* 22:1-25.

11. Weiss HJ, Witte LD, Kaplan KL, Lages BA, Chernoff A, Nossel HL, Goodman DS, Baumgartner HR. (1979) Heterogeneity in storage pool deficiency: studies on granule-bound substances in 18 patients including variants deficient in alpha-granules, platelet factor 4, beta-thromboglobulin, and platelet-derived growth factor. *Blood* 54:1296-1319.

12. Morgenstern E, Reimers HJ. (1985) Fibrinogen distribution on surfaces and in organelles of ADP stimulated human blood platelets. *Eur. J. Cell Biol.* 38:292-300.

13. Komatsu N, Suda T, Sakata Y. (1986) Megakaryocytopoiesis in vivo of patients with essential thrombocytopenia: effects of plasma and serum of megakaryocyte colony formation. *Br. J. Haematol.* 64:241-252.

14. Dickie MM. (1964) *Mouse News Letter* 30:30.

15. Green MC. (1973) *Mouse News Letter* 48:25.

16. Swank R, Jiang SY, Reddington M, Conway J, Stephenson D, McGarry MP, Novak EK. (1993) Inherited abnormalities in platelet organelles and platelet formation and associated altered expression of low molecular weight guanosine triphosphate-binding proteins in the mouse pigment mutant gunmetal. *Blood* 81:2626-2635.

17. Novak EK, Reddington M, Zhen L, Stenberg PE, Jackson CW, McGarry MP; Swank RT. (1995) Inherited thrombocytopenia caused by reduced platelet production in mice with the gunmetal pigment gene mutation. *Blood* 85:1781-1789.

18. von Bruchhausen F, Walter U. (1997) "Platelets and Their Factors," Springer, Berlin. vol.126.

19. Cramer EM, Vainchenker W, Vinci G, Guichard J, Breton-Gorius J. (1985) Gray platelet syndrome: immunoelectron microscopic localization of fibrinogen and von Willebrand factor in platelets and megakaryocytes. *Blood* 66:1309-1316.

20. O'Brien EP, Zhen L, Jiang SY, Novak EK, Swank RT. (1996) High-resolution genetic mapping of the gunmetal gene which regulates platelet production. *Mamm. Genome* 7:206-208.

21. Green EL. Linkage, recombination, and mapping. (1981) "Genetics and probabilities in animal breeding experiments," Macmillan, New York. pp77-113.

22. Detter JC, Nguyen QA, Kingsmore SF. (1998) Identification of novel simple sequence length polymorphisms (SSLPs) in mouse by interspersed repetitive element (IRE)-PCR. *Nucleic Acids Res.* 26:4091-4092.

23. Collins FS. (1992) Positional cloning: let's not call it reverse anymore. *Nat. Genet.* 1:3-6.

24. Dietrich W, Katz H, Lincoln SE, Shin H, Friedman J, Dracopoli N, Lander ES. (1992) A genetic map of the mouse suitable for typing intraspecific crosses. *Genetics* 131:423-447.

25. McCarthy L, Hunter K, Schalkwyk L, Riba L, Anson S, Mott R, Newell W, Bruley C, Bar I, Ramu E, Housman D, Cox R, Lehrach H. (1995) Efficient high-resolution genetic mapping of mouse interspersed repetitive sequence PCR products, toward integrated genetic and physical mapping of the mouse genome. *Proc. Natl. Acad. Sci.* 92:5302-5306.

26. Hunter KW, Watson ML, Rochelle J, Ontiveros S, Munroe D, Seldin MF, Housman D. (1993) Single-strand conformational polymorphism (SSCP) mapping of the mouse genome: integration of the SSCP, microsatellite, and gene maps of mouse chromosome 1. *Genomics* 18:510-519.

27. Kingsmore SF, Barbosa MDFS, Nguyen QA, Ashley JA, Blaydes SM, Tchernev VT, Detter JC, Lovett M. (1996) Physical mapping of the beige critical region on mouse chromosome 13. *Mamm. Genome* 7:773-775.

28. Nelson DL, Ledbetter SA, Corbo L, Victoria MF, Ramirez-Solis R, Webster TD, Ledbetter DH, Caskey CT. (1989) Alu polymerase chain reaction: a method for rapid isolation of human-specific sequences from complex DNA sources. *Proc. Natl. Acad. Sci.* 86:6686-6690.

29. Bennett KL, Hill RE, Pietras DF, Woodworth-Gatai M, Kane-Haas C, Houston JM, Heath JK, Hastie ND. (1984) Most highly repeated dispersed DNA families in the mouse genome. *Mol. Cell Biol.* 4:1561-1571.

30. Krayev AS, Kramerov DA, Skyabin K G, Ryskov AP, Bayev AA, Georgiev GP, (1980) The nucleotide sequence of the ubiquitous repetitive DNA sequence B1 complementary to the most abundant class of mouse fold-back RNA. *Nucleic Acids Res.* 8:1201-1215.

31. Lovett M, Kere J, Hinton LM. (1991) Direct selection: a method for the isolation of cDNAs encoded by large genomic regions. *Proc. Natl. Acad. Sci.* 88:9628-9632.

32. Duyk GM, Kim S, Myers RM, Cox DR. (1990) Exon trapping: a genetic screen to identify candidate transcribed sequences in cloned mammalian genomic DNA. *Proc. Natl. Acad. Sci.* 87:8995-8999.

33. Morgan JG, Dolganov GM, Robbins SE, Hinton LM, Lovett M. (1992) The selective isolation of novel cDNAs encoded by the regions surrounding the human interleukin 4 and 5 genes. *Nucleic Acids Res.* 20:5173-5179.

34. Del Mastro RG, Lovett M. (1997) Isolation of coding sequences from genomic regions using direct selection. *Methods Mol. Biol.* 68:183-199.

35. Detter JC, Mishra VS, Kingsmore SF. (1999) Isolation of mouse transcribed sequences from YAC/BAC clones using an interspersed repetitive element (IRE)-PCR based direct cDNA selection approach. *Nucleic Acids Res.* (submitted).

36. Detter JC, Zhang Q, Mules EH, Novak EK, Mishra VS, Li W, McMurtrie EB, Tchernev VT, Wallace MR, Seabra MC, Swank RT, Kingsmore SF. (1999) Rab geranylgeranyl transferase mutation in the gunmetal mouse causes deficient Rab27 prenylation and platelet synthesis. *Nature* (submitted).

37. Armstrong SA, Seabra MC, Sudhof TC, Goldstein JL, Brown MS. (1993) cDNA cloning and expression of the α and β subunits of rat rab geranyl-geranyl transferase. *J. Biol. Chem.* 268:12221-12229.

38. van Bokhoven H, Rawson RB, Merkx GFM, Cremers FPM, Seabra MC. (1996) cDNA cloning and chromosomal localization of the genes encoding the α- and β-subunits of human rab geranylgeranyl transferase: The 3' end of the α-subunit gene overlaps with transglutaminase 1 gene promoter. *Genomics* 38:133-140.

39. Dietrich WF, Miller JC, Steen RG, Merchant M, Damron D, Nahf R, Gross A, Joyce DC, Wessel M, Dredge RD. (1994) A genetic map of the mouse with 4,006 simple sequence length polymorphisms. *Nat. Genet.* 7:220-245.

40. Kusumi K, Smith JS, Segre JA, Koos DS, Lander ES. (1993) Construction of a large-insert yeast artificial chromosome library of the mouse genome. *Mamm. Genome* 4:391-392.

41. Swank RT, Reddington M, Howlett O, Novak EK. (1991) Platelet storage pool deficiency associated with inherited abnormalities if the inner ear in the mouse pigment mutants muted and mocha. *Blood* 78:2036-2044.

42. Seabra MC, Ho YK, Anant JS. (1995) Deficient geranylgeranylation of Ram/Rab27 in choroideremia. *J. Biol. Chem.* 41:24420-24427.

43. Zhen L, Jiang S, Feng L, Bright NA, Peden AA, Seymour AL, Novak EK, Elliott R, Gorin MB, Robinson MS, Swank RT. (1999) Abnormal expression and subcellular distribution of subunit proteins of the AP-3 adaptor complex lead to platelet storage pool deficiency in the pearl mouse. *Blood* (in press).

44. Deloukas P, Schuler GD, Gyapay G, Beasley EM, Soderlund C, Rodriguez-Tome P, Hui L, Matise TC, McKusick KB, Beckmann JS, Bentolila S, Bihoreau M, Birren BB, Browne J, Butler A, Castle AB, Chiannilkulchai N, Clee C, Day PJ, Dehejia A, Dibling T, Drouot N, Duprat S, Fizames C, Bentley DR. (1998) A physical map of 30,000 human genes. *Science* 282:744-746.

45. Krawczak M, Reiss J, Cooper DN. (1992) The mutational spectrum of single base-pair substitutions in mRNA splice junctions of human genes: causes and consequences. *Hum. Genet.* 90:41-54.

46. Wilson AL, Erdman RA, Castellano F, Maltese WA. (1998) Prenylation of Rab8 GTPase by type I and type II geranylgeranyl transferases. *Biochem. J.* 333:497-504.

47. Kinsella BT, Maltese WA. (1991) Rab GTP-binding proteins implicated in vesicular transport are isoprenylated in vitro at cysteines within a novel carboxyl-terminal motif. *J. Biol. Chem.* 266:8540-8544.

48. Chen D, Guo J, Miki T, Tachibana M, Gahl W. (1997) Molecular cloning and characterization of Rab27a and Rab27b, novel human rab proteins shared by melanocytes and platelets. *Biochem. Mol. Med.* 60:27-37.

49. Sanford JC, Foster L, Kapadia Z, Wessling-Resnick M. (1995) Analysis of the stoichiometry of Rab protein prenylation. *Anal. Biochem.* 224:547-556.

50. Walworth NC, Goud B, Kabcenell AK, Novick PJ. (1989) Mutational analysis of SEC4 suggests a cyclical mechanism for the regulation of vesicular traffic. *EMBO J.* 8:1685-1693.

51. Christoforidis S, McBride HM, Burgoyne RD, Zerial M. (1999) The Rab5 effector EEA1 is a core component of endosome docking. *Nature* 397:621-625.

52. Clague MJ. (1999) Membrane transport: take your fusion partners. *Curr. Biol.* 9:R258-260.

53. Lombardi D, Soldati T, Riederer MA, Goda Y, Zerial M, Pfeffer SR. (1993) Rab9 functions in transport between late endosomes and the trans Golgi network. *EMBO J.* 12:677-682.

54. Gibbs JB, Graham SL. (1995) Inhibitors of geranylgeranyl-protein transferase. US Patent Number US5470832.

55. Seabra MC, Brown MS, Goldstein JL. (1993) Retinal degeneration in choroideremia: deficiency of rab geranylgeranyl transferase. *Science* 259:377-381.

56. Cremers FP, Armstrong SA, Seabra MC, Brown MS, Goldstein JL. (1994) REP-2, a Rab escort protein encoded by the choroideremia-like gene. *J. Biol. Chem.* 269:2111-2117.

57. van den Hurk JA, Hendriks W; van de Pol DJ, Oerlemans F, Jaissle G, Ruther K, Kohler K, Hartmann J, Zrenner E, van Bokhoven H, Wieringa B, Ropers HH, Cremers FP. (1997) Mouse choroideremia gene mutation causes photoreceptor cell degeneration and is not transmitted through the female germline. *Hum. Mol. Genet.* 6:851-858.

58. Swank RT, Novak EK, McGarry MP, Rusiniak ME, Feng L. (1998) Mouse models of Hermansky Pudlak syndrome: a review. *Pigment Cell Res.* 11:60-80.

59. Smith MP, Cramer EM, Savidge GF. (1997) Megakaryocytes and platelets in alpha-granule disorders. *Baillieres Clin. Haematol.* 10:125-148.

60. Yokoyama S, Ohishi N, Shamoto M, Watanabe Y, Yagi K. (1997) Isolation and expression of rat interferon beta gene and growth-inhibitory effect of its expression on rat glioma cells. *Biochem. Biophys. Res. Commun.* 232:698-701.

61. Wei LN, Lee CH, Chinpaisal C, Copeland NG, Gilbert DJ, Jenkins NA, Hsu YC. (1995) Studies of cloning, chromosomal mapping, and embryonic expression of the mouse Rab geranylgeranyl transferase beta subunit. *Cell Growth Differ.* 6:607-614.

62. Cloudman AM, Bunker LE. (1945) The varitint-waddler mouse. *J. Hered.* 36:258-263.

63. Deol MS. (1954) The anomalies of the labyrinth of the mutants varitint-waddler, shaker-2 and jerker in the mouse. *J. Genet.* 52:562-588.

64. Johannes L, Perez F, Laran-Chich MP, Henry JP, Darchen F. (1996) Characterization of the interaction of the monomeric GTP-binding protein Rab3a with geranylgeranyl transferase II. *Eur. J. Biochem.* 239:362-368.

BIOGRAPHICAL SKETCH

John (Chris) Detter was born in Hollywood, Florida, 27 years ago. In 1990, he graduated from Boone High School in Orlando, Florida. Chris initially attended Embry-Riddle Aeronautical University in Daytona Beach, Florida, as an aerospace engineer major. He then went on to complete his undergraduate degree in 1995 at Baylor University in Waco, Texas, receiving a bachelor of science degree in biology. In 1995, Chris entered the Ph.D. program in the Center for Mammalian Genetics at the Department of Molecular Genetics and Microbiology, College of Medicine, University of Florida.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Stephen F. Kingsmore, Chair
Assistant Professor of Molecular
Genetics and Microbiology

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Margaret R. Wallace
Associate Professor of Biochemistry
and Molecular Biology

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Camilynn I. Brannan
Assistant Professor of Molecular
Genetics and Microbiology

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Daniel J. Driscoll
Associate Professor of Molecular
Genetics and Microbiology

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Wayne T. McCormack
Associate Professor of Immunology
and Microbiology

This dissertation was submitted to the Graduate Faculty of the College of Medicine and to the Graduate School and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

August 1999

Dean, College of Medicine

Dean, Graduate School